FINAL REPORT

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Development of biological control for *Alliaria petiolata* (garlic mustard)

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Abstract

Alliaria petiolata, garlic mustard, a biennial plant of European origin accidentally introduced to North America, has spread throughout much of eastern and midwestern North America and is now recorded in 34 states and 4 Canadian provinces. Invasion of natural forest communities by garlic mustard is considered a serious problem because it is believed to displace indigenous herbaceous flora and fauna. Physical, mechanical, and chemical means of management of garlic mustard have failed to achieve long-term control. The development of biological control appears the only viable option for long-term ecologically sound management of the species. Six different beetle species (Chrysomelidae and Curculionidae) were evaluated for their impact and host specificity in Europe. A seed feeding weevil, *Ceutorhynchus theonae*, was too rare, and rearing under quarantine conditions in Switzerland too difficult, to pursue investigations of this insect. A second species, the flea beetle *Phyllotreta ochripes*, showed promise based on multiple generations and significant impact on plant performance. However, the species attacked a number of other plant species, even in multiple-choice feeding tests, and therefore is not considered sufficiently specific to be considered further as a biological control agent.

Detailed investigations focused on the seed-feeding weevil *Ceutorhynchus constrictus*, two stem mining weevils, *C. alliariae* and *C. roberti*, and a stem and root-crown feeding weevil *C. scrobicollis*. The seed feeder was widely distributed in Europe but attack rates remained fairly low throughout the investigative period. This may be, in part, explained by significant mortality through attack by parasitic natural enemies. Experiments showed that the two stem-mining weevils are reproductively isolated species that can co-exist in the same ecological niche. However, their impact on garlic mustard performance in experiments was not particularly dramatic resulting in little reductions in biomass or seed output. Field observations suggest that a larger impact can be expected of these species once released in North America. Problems in experimental design (by limiting the ability of females to move freely among plants) may have created intense competition for oviposition sites. The most promising and significant impact was observed by attack of *C. scrobicollis*, a species active in fall, winter and spring. Attack by this root-mining weevil reduced plant survival, plant biomass and seed output, key variables in plant population demography of garlic mustard.

A test plant list of approximately 50 species taxonomically related to *A. petiolata*, occurring in the same habitat, with chemical similarities, and important agricultural plants were selected for host-specificity investigations. Plants or seeds were obtained in Europe or sent from North America to Switzerland, where they were grown in a common garden. Host specificity investigations were conducted using sequential no-choice tests where females were offered feeding and oviposition sites by alternating test plants and original host (garlic mustard). Species accepted in the fist sequence were tested in no-choice oviposition tests followed by multiple-choice oviposition tests and no-choice oviposition and larval development tests. We encountered significant problems in synchronizing

North American plants with insect activity periods. An additional problem was the abundance of naturally occurring polyphagous insects that attack species in the Brassicaceae in Europe. We had a constant need to protect host plants grown in a common garden from this attack, which could be accomplished by using cages, pesticide, or growing plants in the greenhouse. All of these methods have drawbacks, never work 100%, and may influence the quality of test plants, which may result in findings that represent lab artifacts rather than a predictive test.

We were unable to complete the entire sequence of all 50 plant species proposed for testing during the 3 years of this project, although the vast majority of plants was tested for all 4 weevil species currently considered as potential biological control agents. All species accepted a number of species in the Brassicaceae for oviposition in sequential no-choice tests but only few test plants allowed larval development. *Brassica nigra* was the only test plant species that allowed larval development *of C. constrictus*, and *C. alliariae* and *C. roberti* adults emerged from *Thlaspi arvense* and *Nasturtium officinale*. Both of these plant species are introduced to North America and considered weeds, at least in part of their distributions. The root feeder *C. scrobicollis* is the most specific of the 4 species and confirmed development of a single adult occurred in only a single test on *Brassica oleracea sabauda* in one year, and this result could not be repeated with other varieties, indicating that this may have been an artifact of testing conditions. In addition, this particular variety is not grown in North America. A significant problem in the selection of test plant species was the recent taxonomic rearrangements within the Brassicaceae and we have incorporated additional species into our sequence that were not part of the original list but now appear closely related to the target weed. As an additional safety precaution, we will need to complete additional test in Europe and under quarantine in North America for a number of North American Brassicaceae. We have been able to get funding support from the US Forest Service for this work and additional tests are planned for a newly opened facility in Minneapolis, MN beginning in 2003. A team will visit CABI in March 2003 to learn methods of testing and rearing and the initial focus will be *C. scrobicollis*.

Surveys for potential natural enemies in North America were conducted throughout the range of the species. We developed a standardized sampling protocol and asked collaborators to send samples to Cornell University. We received samples from 49 locations in North America and dissected and recorded attack on over 6000 garlic mustard stems. The most common species encountered were a leaf-mining fly, a stem-mining weevil, a stem-mining fly, and a number of externally feeding species such as spittlebugs. Attack rates were generally extremely low, with the exception of an outbreak of the leaf miner in the Midwest in 2001 with hundreds of mines/0.25m^2 at certain sites. A polyphagous fungus killed a large number of plants at a field site in the Northeast in 2000 but this attack had no lasting effect on abundance of garlic mustard at that particular site. The stem mining weevil was more common in the Northeast but attack rates were usually well below 5% of stems. All species encountered were attacked by their own suite of natural enemies (hymenopterous parasitoids). All herbivore species were sent for identification but no confirmation of species identity has been received. It is possible that a number of these species represent accidental introductions from Europe. Rearings of two weevil species commonly encountered on *A. petiolata* in the Northeast showed that while adults can survive on garlic mustard, fecundity is greatly reduced and this species is not the primary host plant for these weevils.

In preparation for a potential introduction of insects for biological control of garlic mustard, we developed a standardized monitoring protocol. This protocol incorporates measures of control agent abundance and feeding, garlic mustard performance (height, number of stems and siliques, number of rosettes, cover), and the response of the associated plant community in permanent $0.5m^2$ plots. This protocol was tested at 4 field sites (2 in Illinois, 2 in New York) and a workshop conducted in June 2003 introduced 30 natural area managers to the procedures. Comments by the participants were incorporated into the current draft of the protocol and additional workshops are planned for the Midwest in June 2003. Our monitoring showed that while the community (largely composed of perennial plants) remains stable at each of the 4 monitoring sites, garlic mustard abundance fluctuates greatly from year to year. These fluctuations can be explained, in part, by the biennial life cycle of the plant, but also by variable garlic mustad recruitment and survival in response to climatic conditions. Any implementation of a monitoring program has to contend with these annual variations and only long-term monitoring will be able to detect a sustained impact of the release of biological control agents. Ideally, monitoring should be implemented a few years before releases are actually carried out. After the development of our protocol, a number of managers have begun establishment of permanent monitoring quadrats in anticipation of a potential introduction of insects, and we receive continued requests for the protocol. A version of the protocol will be available in pdf-format at the website of the Ecology and Management of Invasive Plants Program (www.invasiveplants.net) at Cornell University.

While we were unable to complete the entire testing sequence for all control agents, we are well on our way to complete all host specificity work and to file a petition with TAG within the next 12-15 months (at least for *C. scrobicollis*). Two of the six species initially under investigation are not specific enough to be considered potential control agents but the remaining four species show great promise for implementation of garlic mustard biocontrol. In particular the root feeder *C. scrobicollis* appears to be a species with considerable potential to reduce performance and abundance of *A. petiolata*. We are confident that the remaining tests with additional North American plant species will show the specificity of all agents under investigation. With the development of a standardized monitoring protocol before introduction of control agents, we have made great progress in implementing a long-term monitoring program that will be important in evaluating the success and safety of biological control of garlic mustard.

Introduction

Alliaria petiolata, garlic mustard, a biennial plant of European origin, was accidentally introduced to North America and was first recorded in 1868 on Long Island, NY (Nuzzo 1993). The species has spread throughout much of eastern and midwestern North America and is now recorded in 34 states and 4 Canadian provinces (Nuzzo 1993, Blossey et al. 2001, Blossey et al. 2002). Garlic mustard readily colonizes disturbed forest communities and primarily disperses along corridors of both naturally and anthropogenically disturbed habitats (Nuzzo 1993, Nuzzo 1999). Invasion of natural forest communities by garlic mustard is considered a serious problem because it is believed to displace indigenous herbaceous flora and fauna (Blossey et al. 2002). Several methods have been used to control the proliferation of garlic mustard in natural areas. Hand removal can be effective in small infestations, and fire, cutting, and herbicide treatments have been used to reduce garlic mustard densities in large infestations. However treatments need to be repeated over many years to deplete the seed bank, they are disruptive to many native organisms and, over large areas, prohibitively expensive (Blossey et al. 2001). Many military installations in the Northeast, Southeast, and Midwest have applied physical, mechanical, and chemical means of management against garlic mustard but, as in many other instances, have failed to achieve long-term control. The development of biological control appears the only viable option for long-term ecologically sound management of garlic mustard (Blossey et al. 2001).

Objectives

Initial fieldwork and a feasibility study in Europe revealed 69 phytophagous insect species and seven fungi associated with garlic mustard in Europe (Hinz and Gerber 1998). Literature records and field observations suggested that the potential for development of biological control for garlic mustard appeared promising and a 3-year project funded by SERDP continued and extended the preliminary work funded through various other sources. Work to be accomplished under this grant consisted of investigations in Europe and North America and had the following 2 main objectives:

<u>Objective 1</u>
Study ecology, life history and impact of potential biocontrol agents for garlic mustard in Europe and determine their host specificity.

<u>Objective 2</u>
Develop and test a standardized monitoring protocol for follow-up studies at select monitoring sites that will also serve as initial field release sites for potential biocontrol agents.

Technical Approach

Personnel at CABI Bioscience Center Switzerland studied ecology, life history, impact, and specificity of 6 potential biocontrol agents for garlic mustard in Europe. These studies were intended to reveal details of the life history (phenology, competitive interactions, natural enemies) and impact on plant growth and population dynamics of potential biological control agents. Multiple herbivore impact (attacking seeds, shoots and roots) is assumed to lead to accelerated performance reductions in plants (compared to attack by a single species), but investigations were conducted to exclude the potential for competitive interaction among the different potential biocontrol agents attacking garlic mustard. Scientists have speculated that such competitive interactions (particularly of root feeders and folivores) may have the potential to reduce the overall success of a weed control program, however, available evidence from past control programs does not support these claims (Blossey and Hunt-Joshi 2003). Nevertheless, studies were conducted to determine whether introduction of multiple agents to North America is warranted or should be avoided.

Determining the host specificity of potential control agents is of overriding importance before importation of biocontrol organisms can be approved by USDA. Personnel at CABI investigated the host specificity of potential biological control agents of garlic mustard using feeding, starvation, and oviposition tests with adults and larvae in the laboratory, on potted plants and in the field.

After release of biocontrol agents, natural area managers anticipate population reductions of garlic mustard, a return of native plant communities and associated fauna. Past evaluations of biocontrol programs have suffered from lack of standardization of protocols and lack of scientific rigor (Blossey 1995, Blossey 1999, Blossey and Skinner 2000). To allow an appropriate assessment of the anticipated impacts of a release of biocontrol agents on garlic mustard and native plant communities, we developed a standardized monitoring protocol. This work was conducted in North America in cooperation with Victoria Nuzzo, Natural Area Consultants. As part of this protocol development, we studied the insect communities on garlic mustard in North America, and used field visits in Europe to get a first hand impression of feeding damage and visibility of the organisms in the field. The protocol now incorporates measures of garlic mustard performance (height, seed production) and abundance (presence/absence, number of stems, cover). Permanent field sites were established in New York (West Point Military Academy, Ithaca) and Illinois (Fermi Lab, Rockford) and preliminary versions of the monitoring protocol were tested for several years.

Work in Europe

Based on information on their restricted host-range and their damage, five weevils and one flea beetle (Table 1) were selected as potential biological control agents for garlic mustard, and their life history and ecology were investigated in Europe. A full list of organisms attacking garlic mustard in Europe can be found in Hinz and Gerber (1998).

Table 1: Potential biocontrol agents associated with *Alliaria petiolata* in Europe

Species	Plant structure attacked
Coleoptera, Curculionidae	
Ceutorhynchus constrictus	seeds
Ceutorhynchus theonae	seeds
Ceutorhynchus roberti	stems, petioles
Ceutorhynchus alliariae	stems, petioles
Ceutorhynchus scrobicollis	stems, roots, rosettes
Coleoptera, Chrysomelidae	
Phyllotreta ochripes	roots

Throughout the growing season from 2000-2002 in Europe, field surveys and collection trips were conducted in Germany, Switzerland, Austria, and Italy to obtain specimens for life history, host specificity, and impact investigations, as well as for assessing impact and population densities of various biocontrol agents and their natural enemies.

Ceutorhynchus constrictus

Life history

Ceutorhynchus constrictus (Marsham) is a univoltine weevil. It is the most widespread of the *Ceutorhynchus* species associated with garlic mustard and is commonly found all over Western and Central Europe (Dieckmann 1972). In Central Europe, adults appear in April to feed on leaves and mate. Oviposition starts once *A. petiolata* begins to produce siliques (seed pods) in May and June and eggs are laid into developing seeds. A single female may produce well over 150 eggs during a season. Larvae feed on developing seeds with each larva consuming 2-3 seeds (2.5±0.34, mean ±SE) before leaving the silique to pupate in the soil. Mature larvae form an earthen cocoon, pupate, and fully developed adults overwinter but delay emergence until the following spring.

The phenology of *C. constrictus* was investigated in the common garden at CABI and plants were exposed to ovipositing females from 12-18 June 2001. Plants were dissected in 4 day intervals and the occurrence of different larval stages recorded (Fig. 1). As expected, the phenology was quite synchronized, with the larval cohort passing through the various stages synchronously and completing development in about 30-40 days. Eggs needed over a week to develop into larvae but the remaining development was rather fast. In the field, a more asynchronous development can be expected since plants may be flowering and fruiting for several weeks.

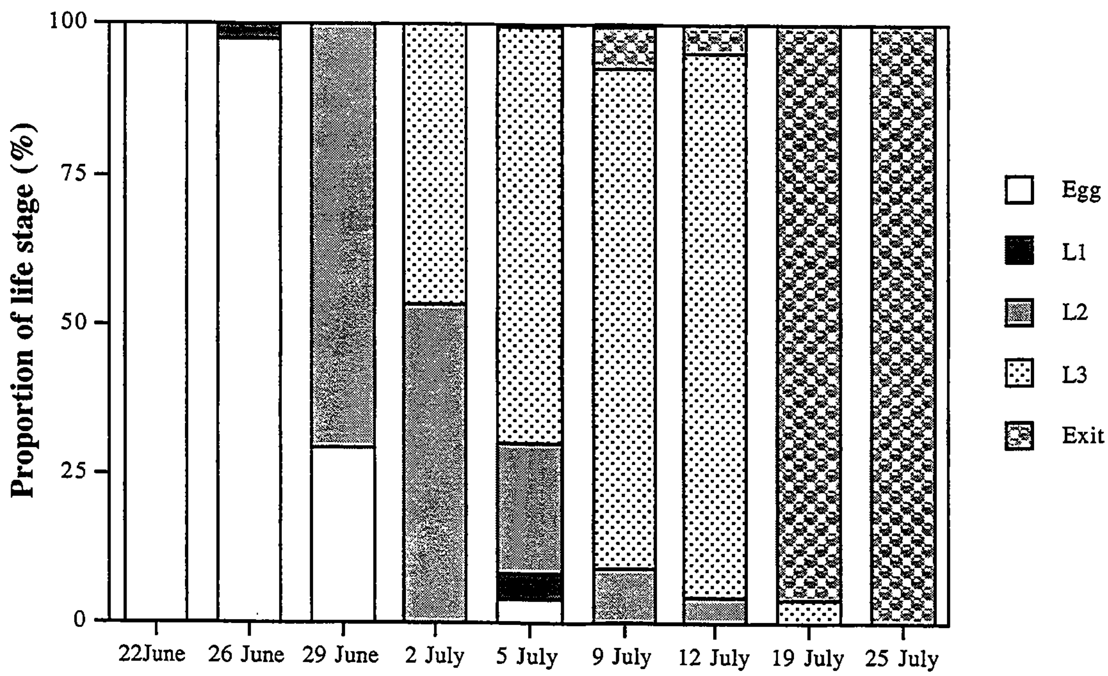

Fig. 1. Phenology of immature stages of *C. constrictus* on plants kept in a common garden in Delemont, Switzerland. Females were allowed to oviposit onto garlic mustard plants from 12-18 June 2001 and plants dissected in 4-6 day intervals thereafter until 25 July 2001. The category "exit" refers to exit holes found in the stems. We record each exit hole as a single larva leaving the stem to pupate in the soil. (N=284 observations).

Impact

Although the species was found at all field sites in our surveys, attack rates were generally low with only 0.3-6.4% of seeds attacked in southern Germany and Switzerland.

Natural enemies

The ectoparasitoid *Trichomalus perfectus* (Hymenoptera, Pteromalidae) was reared on several occasions from third instar *C. constrictus* larvae. Attacked larvae were first found in plants sampled in late June. A more extensive project associated with the Agricultural Section of CABI investigated the field host-range of parasitoids attacking the cabbage seedpod weevil, *Ceutorhynchus obstrictus* (Marsham) (=*C. assimilis* (Paykull)). This species is a serious pest of canola and has been introduced to North America. The Agricultural section of CABI is investigating the potential for biological control of this insect pest. As part of these investigations larger samples were taken from *Ceutorhynchus* species attacking garlic mustard and parasitoids encountered were reared and identified. After several seasons it now appears that the first parasitoid larvae attacking *C. constrictus* are encountered in late June/early July. Attack rates are usually low, differ between field sites, but can reach 15% in early July. Adults of *C. constrictus* were collected from various field sites in the vicinity of Delémont in late April/early May 2002 and regularly checked for adult parasitoids. None of the 60 adults kept for 3-4 weeks was attacked. More field sites and larger quantities of adults need to be checked before we can assume that the species is not attacked by any adult parasitoids.

Ceutorhynchus theonae

This newly described species was discovered in Daghestan, Russia in spring 2000 and shipments into quarantine at CABI, Switzerland were arranged. Preliminary investigations confirm that the species attacks seeds of garlic mustard. The biology of *C. theonae* appears similar to *C. constrictus*, however, larval feeding by *C. theonae* appears to be more damaging compared to *C. constrictus*, i.e. more seeds are consumed/larva. A new shipment of beetles from Daghestan arrived in spring 2001 at CABI; unfortunately, all adults were dead on arrival. A small rearing of this species existed in quarantine, however, the difficulties in obtaining sufficient insect material and the elaborate care this colony requires while maintaining it in quarantine, will not allow in-depth investigations of this species. Efforts to rear and test this species were terminated in 2001 and no host specificity tests were conducted. The species may remain of interest in the future in case it is determined that introduction of an additional seed feeding species is warranted.

Ceutorhynchus alliariae and *Ceutorhynchus roberti*

Life history

The two weevil species *Ceutorhynchus alliariae* and *C. roberti* share similar life-history features and occupy the same ecological niche on their host plant. Adults feed on leaves; larvae develop in stems and leaf petioles of garlic mustard. *Ceutorhynchus alliariae* and *C. roberti* appear to differ in their geographic distributions with only *C. alliariae* occurring in Northern and Eastern Germany and Eastern Austria and *C. roberti* as the only species reported from Italy (Abazzi and Osella 1992). However, both species overlap in their distribution in southern Germany and Switzerland. Adults of both species overwinter in soil and leaf litter, and become active simultaneously in early spring. In Europe, oviposition begins in March and continues through May and there is little difference in oviposition phenology between the two species (Fig. 2).

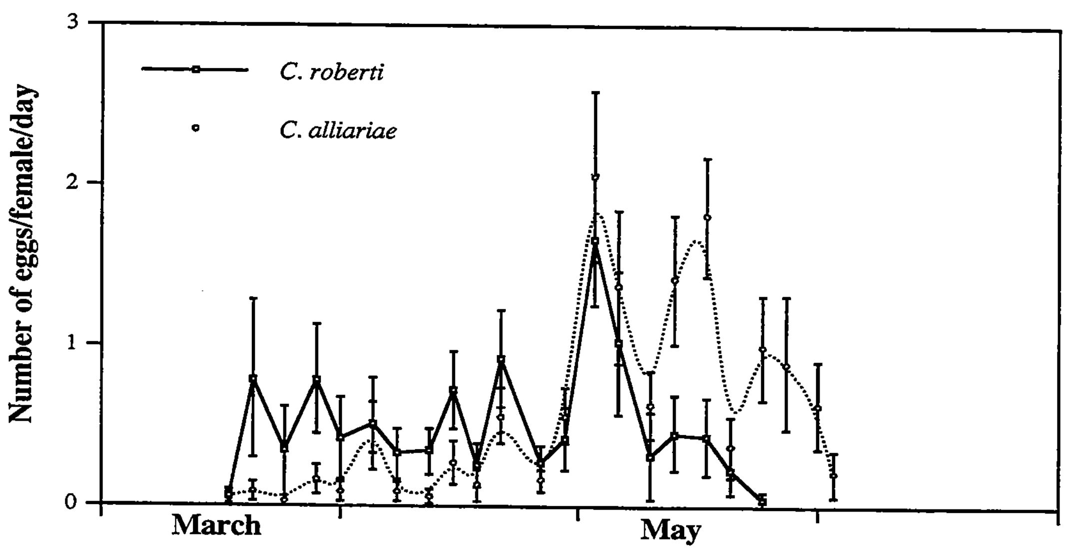

Fig. 2. Seasonal oviposition pattern in spring 2001 of *Ceutorhynchus roberti* (squares)and *C. alliariae* (circles)on cut shoots under ambient temperatures. Data are means ±SE of 7 replicates/species. Data from twice-overwintered females during their second oviposition period.

New evidence indicates that adult weevils of both species appear to be long-lived (Fig. 3) and can have multiple successful oviposition periods (Figs. 4, 5). Adults emerging in summer 1999 from field collected stems overwintered several times at CABI. Although survival was highest (>80%) for their first overwintering and almost 50% of the adults died during the spring/summer season in 2000, between 60-77% of the survivors overwintered successfully a second time (Fig. 3). Substantial mortality occurred during and after the second oviposition period, yet almost 10% of adults emerged in 1999 were still alive in the fall 2001 (Fig. 3) and were overwintered at CABI. There was no obvious difference in survival rates between males and females or between the two species during this period. In spring 2002, 4 individuals of *C. alliariae* and two *C. roberti* adults were still alive and tested for their oviposition. All individuals died by the end of June 2002.

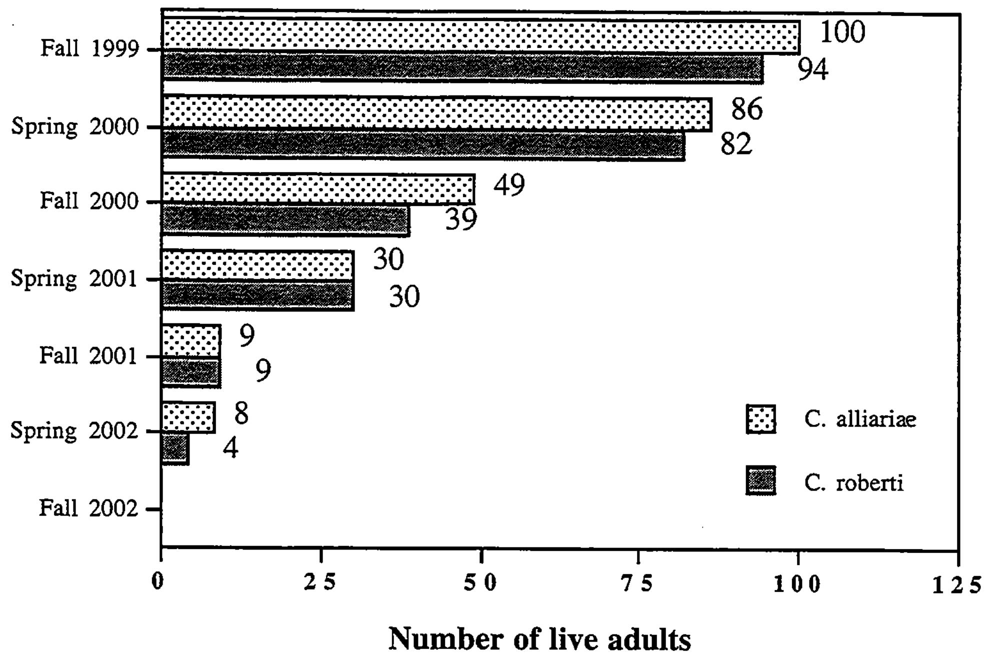

Fig. 3. Number of surviving adults of *C. alliariae* and *C. roberti* from emergence in summer 1999 through fall 2001.

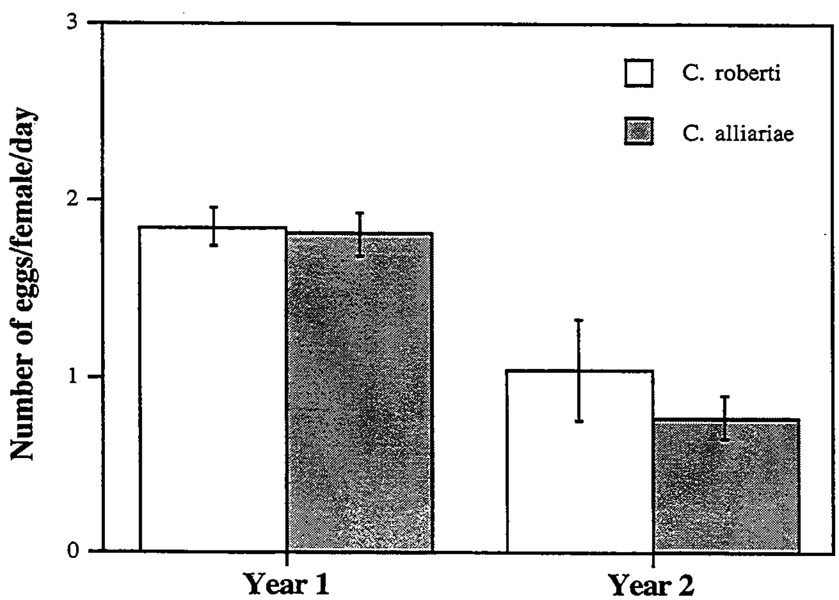

Fig. 4. Number of eggs/female/day laid during two separate oviposition periods of *Ceutorhynchus roberti* and *C. alliariae*. Data are means ± SE of 7-10 replicates/ species/ year.

Females produced fertile eggs in their first and second year of oviposition, however, the daily oviposition rate (Fig. 4) and the overall fecundity in the second year is reduced by nearly 50% (Fig. 5) for both species. The number of eggs produced per female/day is identical for *C. roberti* and *C. alliariae* females in their first and second year of oviposition (Fig. 3). Surviving females had a third oviposition period and larvae hatched successfully, although the number of eggs was greatly reduced (Fig. 5). The three *C. alliariae* females laid 1, 47, and 71 eggs respectively; the only *C. roberti* female laid 5 eggs.

In spring 2001 a mark-recapture experiment was conducted to estimate population size in a field site close to Delémont 6. A total of 41 weevils (18 *C. alliariae* and 23 *C. roberti*) were captured, marked on the elytra with a spot of white nail varnish, and released. Nearly a year after marking adults, the field site was visited occasionally between 27 February and 13 May 2002 to investigate whether adults survived extended periods under field conditions. Three marked *C. alliariae* (1 female, 2 males) and seven *C. roberti* (2 females, 5 males) were collected, and one female of each species laid viable eggs. This confirms that both species are longlived and can have at least a second oviposition period under field conditions.

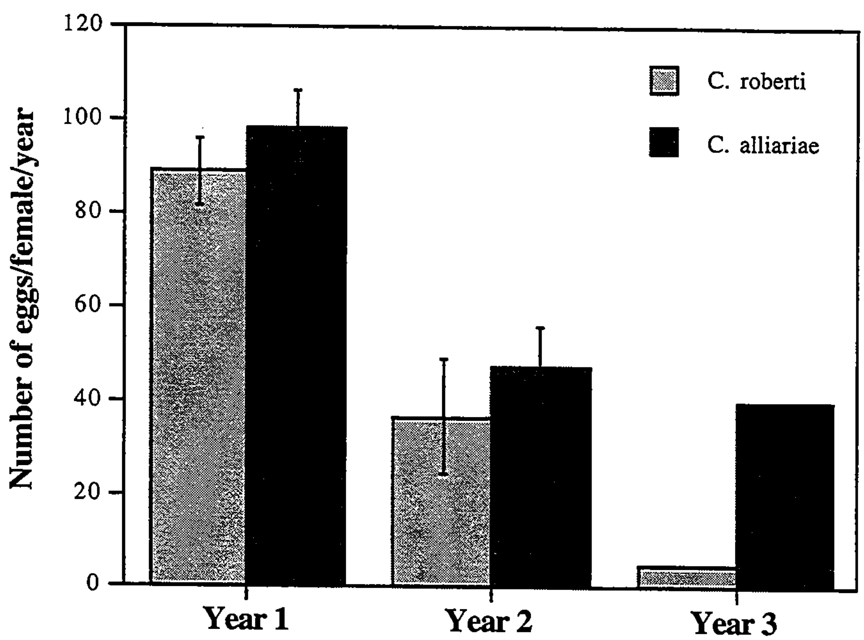

Fig. 5. Number of eggs/female/year laid during three separate oviposition periods of *Ceutorhynchus roberti* and *C. alliariae*. Data are means ± SE of 7-10 females/ species/ year in year 1 and 2. Only 1 *C. roberti* and *3 C. alliariae* females were available in year 3.

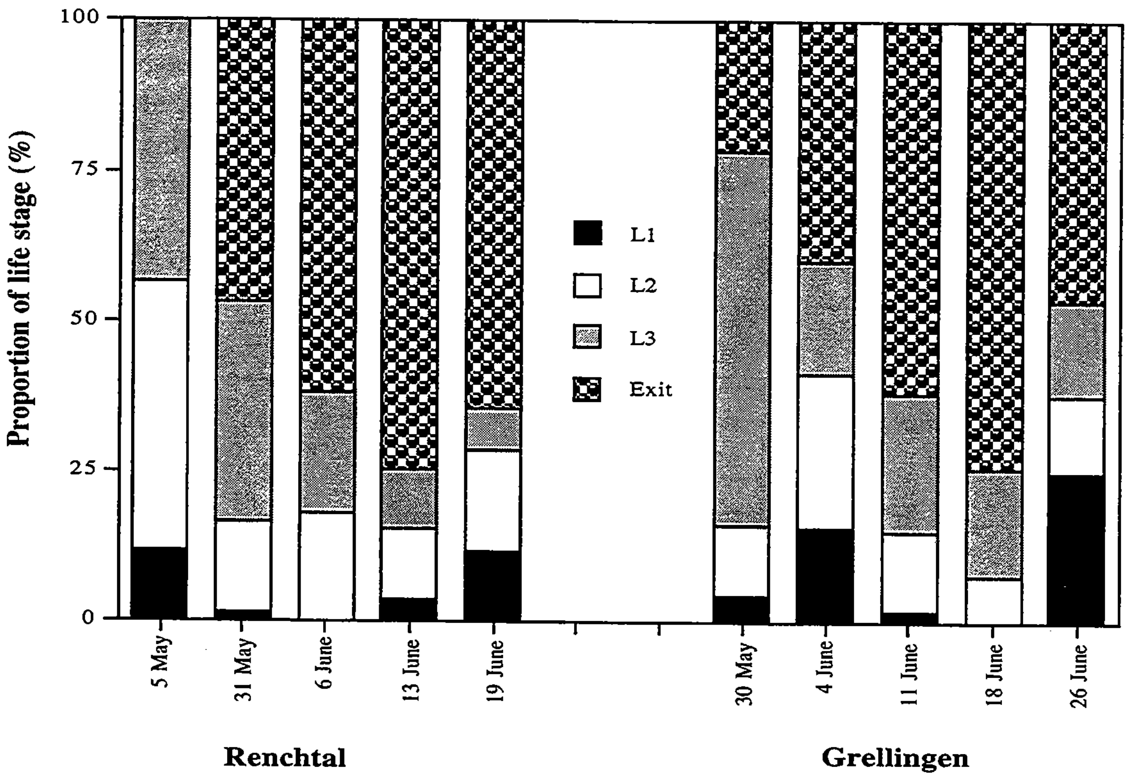

Fig. 6. Phenology of attack and of life stages of *C. roberti* and *C. alliariae* (species inseparable) at two field sites in southern Germany (Renchtal) and western Switzerland (Grellingen).

Larvae of both species hatch after 2-3 weeks and feed internally in stems of garlic mustard. Mature third instar larvae leave the host plant to pupate in the soil. At the time of oviposition, rosettes of recently germinated garlic mustard seeds are present at many field sites. Work in the laboratory and dissections of (large) rosettes collected in the field revealed that both *C. roberti* and *C. alliariae* will oviposit and feed in leaf petioles of rosettes. In our experiments larval survival was low but attack rates were occasionally higher (per unit volume of the stem) in rosettes than in bolting plants (potentially a function of the softness of petiole tissue). This suggests that both species may significantly contribute to rosette mortality. Whether the species are able to successfully develop to adults in rosettes routinely, or whether rosette attack is a population sink, will need to be investigated in more detail (see also under natural enemies). Larval development from egg to mature larvae takes about 7 weeks with new generation adults emerging in June and July. At two field sites in southern Germany and western Switzerland, the attack rates, phenology of attack and life stages were investigated in spring 2001. Random stem samples were dissected and the number of eggs, 1st-3rd larval instars, and exit holes were counted between early May and mid June (Fig. 6).

In early May the majority of larvae reach the second and third instar and by end of May larvae have start to leave the stems for pupation in the soil (Fig. 6). The long oviposition period (Fig. 2) creates a wide size range and asynchronous larval development, however, the absence of eggs in early June indicates that oviposition has largely ceased. The re-occurrence of eggs by mid June is either an indication that some females have extended oviposition periods (only green plants were collected at this time in the season) or that early emerging new generation adults have started to lay eggs before overwintering. (This is a common phenomenon in insects with early emerging teneral adults producing a partial second generation. Experimental studies need to be conducted to tease apart contributions of overwintered and teneral beetles to this "flush" of new larvae).

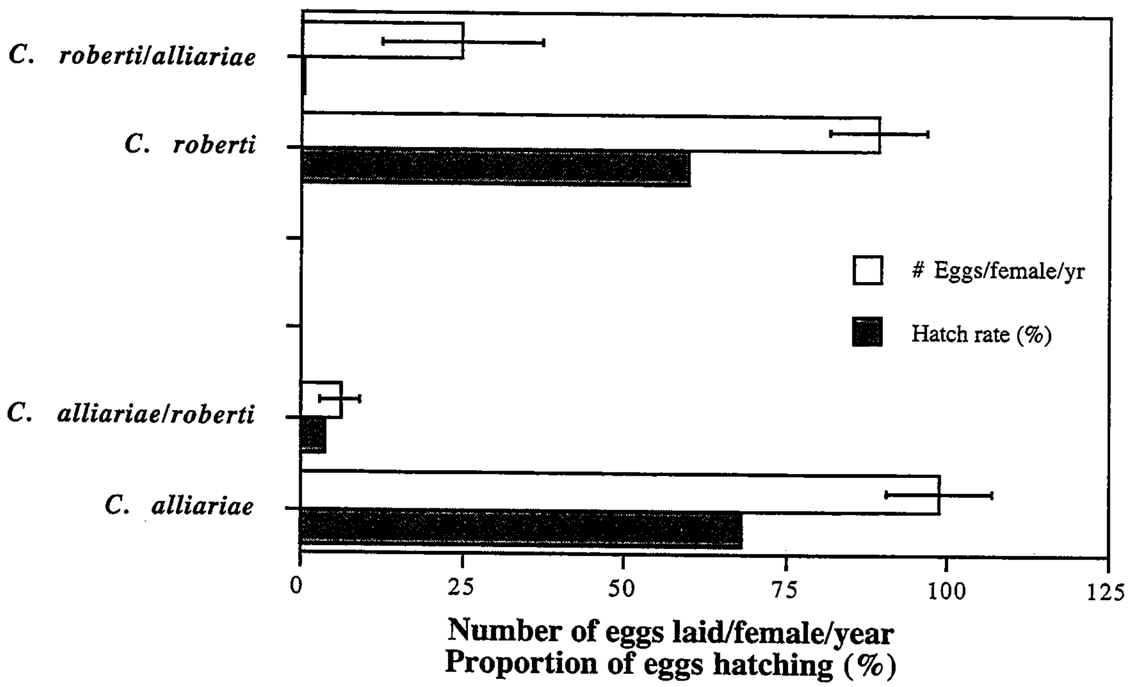

Fig. 7. Number of eggs produced/year of females of *C. roberti*,*C. alliariae* and mixed species pairs and their respective hatch rates. *C. roberti/alliariae* indicates females of *C. roberti* kept with males of *C. alliariae*, *C. alliariae/roberti* indicates females of *C. alliariae* kept with males of *C. roberti*. Data are means of 5 replicates ±SE for eggs/female/year and proportion of all eggs laid during the season that hatched.

<u>Coexistence, competition and impact</u>

While in the past, *C. alliariae* was considered a subspecies of *C. roberti*, presently their species status is generally accepted (Dieckmann 1972). *C. alliariae* lays all eggs individually, while *C. roberti* oviposits eggs either individually (60%) or in clusters of up to 8 eggs (40%) into elongating stems and leaf petioles of garlic mustard. A cross breeding experiment established at CABI (newly emerged females of either species were kept with males of the other species for overwintering and during a complete oviposition period) now supports the latest taxonomic treatments (based on tarsus coloration and penis structure). The number of eggs produced during an oviposition period is drastically reduced in mixed species pairs, and the hatch rate is even more dramatically reduced (Fig. 7). (It can not be completely excluded that some of the eggs found upon dissection of plant material had previously been laid by "wild" females since potted plants were kept outside. See host specificity section for a discussion of "contamination" problems).

Attack rates in the field

Attack rates in the field remain high throughout the study period. At sites investigated in May/June 2000/2001 attack was usually >75% (Fig. 8) and there was no obvious difference between northern and southern sites. Attack of the two species was impossible to separate during dissection but at the northernmost sites in Lübeck, only *C. alliariae* was present. This indicates that high attack rates can be achieved by a single species alone. The average number of larvae that attacked each plant varied among sites but ranged from 1->26. The average number of larvae per shoot ranged from 1-9 during the 2001 field season.

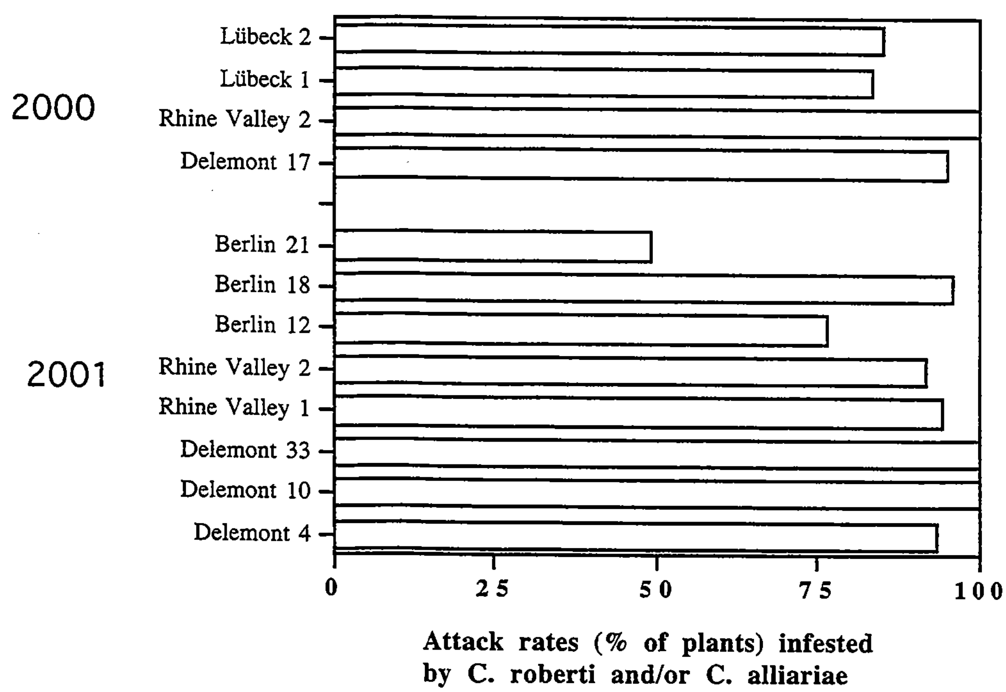

Fig. 8. Attack rates (% of plants) of *C. roberti* and *C. alliariae* (species inseparable) at field sites in western Europe during 2000 and 2001 field seasons. Data were collected in May/June of each year by collecting and dissecting random samples (N=7-70 plants/site). Sites in each year are arranged from North to South.

Competition/impact experiment with C. *alliariae*/C. *roberti*

The two weevil species *C. alliariae* and *C. roberti* share the same ecological niche on their host plant garlic mustard. Although their distributions in Europe are different, both species co-occur in a large geographic region and occur at the same field sites. We established a common garden experiment to investigate experimentally whether the two species compete with each other by releasing pairs of adults in varying densities onto caged host plants. The same densities (of a single species) were established as a control. A competitively inferior species should show reduced survival and recruitment in treatments were it was forced to co-exist with the second species compared to treatments where it was allowed to exploit the resource without competition.

A second objective of this experiment was to assess the impact of different densities of these two potential control agents on host plant performance and reproduction. The goal of the biocontrol program targeting garlic mustard is to reduce the competitive ability of the plant and reduce its reproduction. Therefore, we should select the most damaging species. We hypothesize that with increasing densities of adults, plant performance and reproduction should decrease. Comparing the impact of single or multiple species at various densities provides useful data for determining whether to introduce a single (which one?) or both species (provided they are sufficiently host specific).

Methods

In March 2000, 140 plants of similar size were selected for the experiment from rosettes available at CABI. These plants had been grown from seed collected in the vicinity of Delémont and been protected from herbivory in field cages. However, unexpectedly, some of the plants had been attacked by *C. roberti* occurring naturally in the garden at CABI. Therefore all early spring shoots were removed between 28-30 March, all plants were searched for weevils and the upper soil layer removed (where adults tend to hide), and each plant was covered individually with a gauze bag to protect them from further attack. Ten plants (30 plants for the control, i.e. no weevil release) each were randomly assigned to one of 12 treatments (Table 4). The number of shoots per plant and shoot height were measured, and each plant visually assigned into one of 3 size classes (small, medium, large). On 13 April, a common garden with pots dug into the ground about 50 cm apart from each other, and arranged in five rows (28 pots per row), was established. Within each row treatments were assigned randomly and each treatment occurred twice in each row. Plants were individually covered with gauze bags (55 cm diameter, 150 cm high) using elastic to secure the gauze to the pot. Each bag was kept upright by attaching it to a wire running across the row. A wire ring (30 cm diameter) was attached at the upper end of the gauze bag for stabilization and to spread the gauze.

Table 2. Experimental design for analysis of impact on garlic mustard of and competition between *C. roberti* and *C. alliariae* at various insect densities

Treatment	Pairs of *C. roberti*	Pairs of *C.alliariae*	Total # of pairs
1	0	0	O
2	1	0	1
3	0	1	1
4	2	0	2
5	0	2	2
6	1	1	2
7	4	0	4
8	0	4	4
9	2	2	4
10	8	0	8
11	0	8	8
12	4	4	8

In spring 2000, adults of *C. alliariae* and *C. roberti* were collected from garlic mustard in the vicinity of Delémont. To verify that females were fertile, a single pair was placed into a small transparent plastic cup (6.5 cm diameter, 7 cm high) covered with a gauze lid, and offered a cut leaf (*C. alliariae*) or whenever possible a cut shoot (*C. roberti*) of garlic mustard. After 2-3 days the petioles and shoots were dissected for eggs. Only females that laid eggs were used in the experiment. Weevils were released in a staggered fashion with the first half of all replicates in each row receiving weevils on 14 April 2000, and the second on 24 April 2000.

Number of shoots per plant, height of each shoot, and number of dead main and secondary shoot tips was recorded on 22 May 2000. Adult feeding was quantified by visually assigning plants to four attack levels: no, low, medium and high. Shoots of all plants were cut just above the root crown between 14 and 19 June 2000, transferred separately for each plant into plastic bags and stored at 2 °C until dissection. The root systems and soil of each plant were placed into adult emergence traps. Weevils were collected twice per day and the species, number and sex recorded. All females were weighed and kept separate according to treatment in rearing cylinders.

All shoots were subsequently dissected and the number of shoots per plant, shoot height and base diameter, number of dead and alive larvae, number of dead, alive, and flowering inflorescences, number of viable pods and seeds per shoot were recorded. In addition, each shoot was assigned into one of four attack levels: no, low, medium and high larval mining. Larvae found alive were transferred into soil for pupation. By the end of August weevil emergence had ceased and roots were removed from each pot, cleaned and shoots and roots were dried for 24 hours at 70 °C and their biomass determined.

Data Analyses

Data were analyzed using two way ANOVAs with weevil density and species composition as factors. Polynomial contrasts were used to assess the relationship of weevil density and plant parameters.

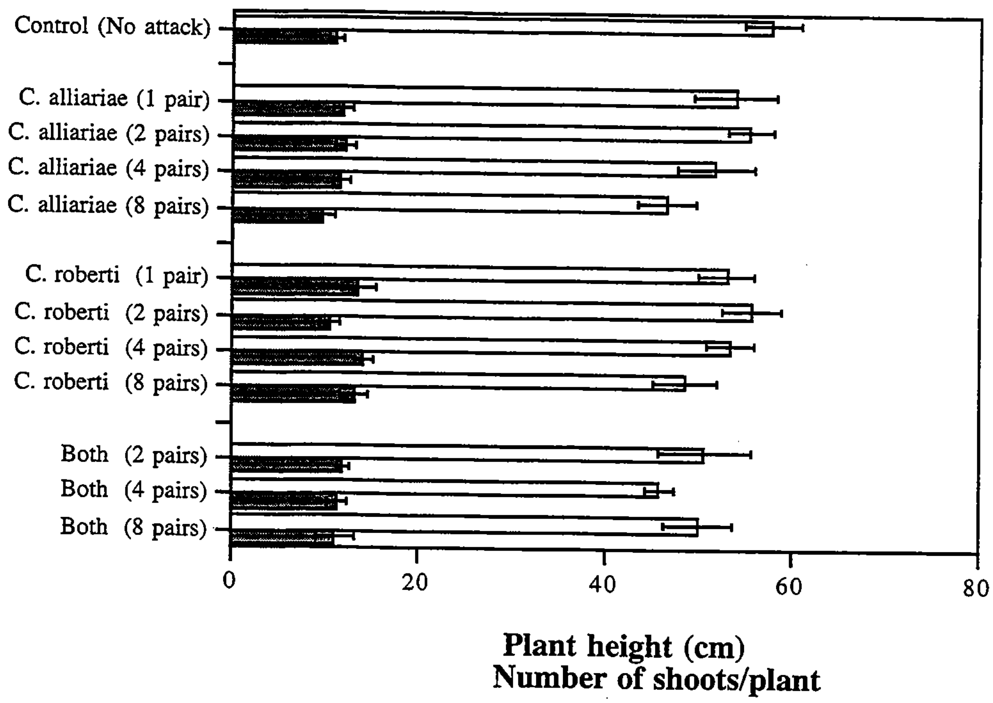

Fig. 9. Impact of weevil density and composition on height (open bars) of garlic mustard and number of shoots (solid bars) per plant. Data are means ±SE of 7-10 replicates/treatment.

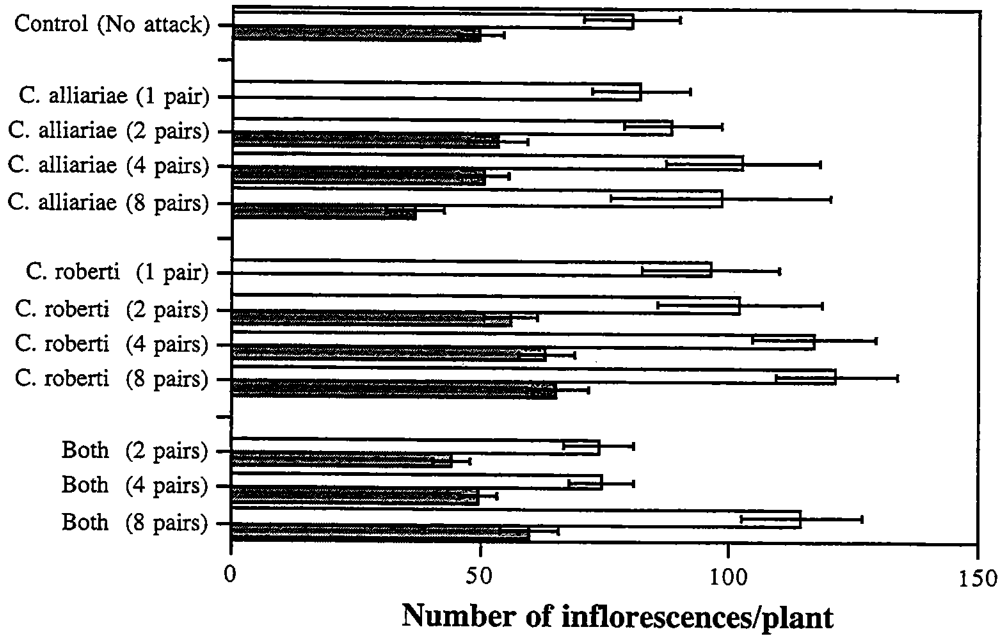

Fig. 10. Impact of weevil density and composition on total number of inflorescences (open bars) of garlic mustard and number of inflorescences producing seed (solid bars) per plant. Data are means ±SE of 7-10 replicates/treatment.

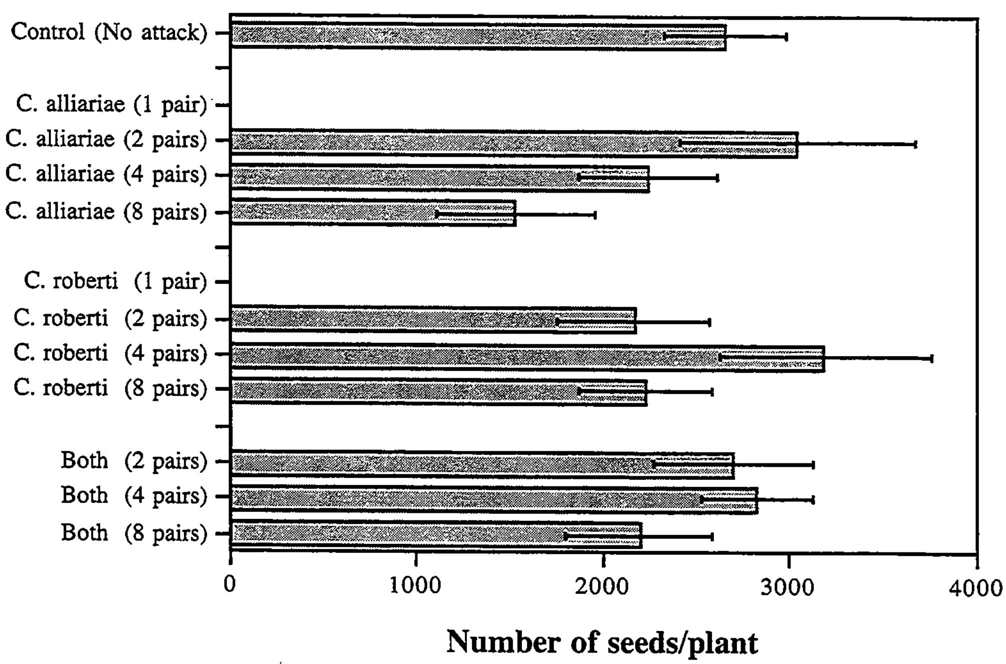

Fig. 11. Impact of weevil density and composition on number of seeds produced per plant. Data are means ±SE of 7-10 replicates/treatment.

Results and Discussion

C. roberti appeared to be more sensitive to handling with a few plants failing to be attacked at all (which were excluded from the analyses). Overall the applied treatments showed no increased mining intensity (visually scored in categories of no, low, medium, high) as a function of increased weevil density (data not shown). While the control plants remained unattacked, plants in all herbivore treatments showed a similar medium-high mining intensity. Plant performance was often significantly influenced by weevil density with little difference due to weevil identity or composition. Plant height decreased slightly (approx. 10cm) but significantly with increasing weevil density (Fig. 19) and this pattern was not different among single and mixed species treatments. The number of shoots/plant was not significantly different among the treatments (Fig. 9). The mean number of inflorescences/plant increased with increasing weevil density (Fig. 10). The number of inflorescences/plant producing viable seeds increased with increasing density of *C. roberti* and the combination of *C. roberti* and *C. alliariae*, while it decreased with increasing density of *C. alliariae* (Fig. 10) The interaction between weevil density and species composition was therefore also significant. The mean number of seeds produced per plant was reduced (marginally significant) with increasing weevil density, independent of species composition. (Fig 11). Plants onto which eight weevil pairs had been released produced approximately 25% less seed compared to unattacked plants.

One goal of this combined impact/competition experiment was to investigate potential competitive interaction among the two species. We achieved the goal to create intense interspecific and intraspecific competition with our experimental design. In fact, our design resulted in significant mortality during egg and larval stages and only minor recruitment (Fig. 12). Thus, the design should allow to detect the presence of any interspecific competition. However, the mean number of offspring produced per female was only significantly reduced as a result of increased weevil density (Fig. 12). Neither species was competitively superior, in fact, symmetrical inter- and intra-specific competition occurred, i.e. both species were equally negatively influenced by increased density of females of the same or of the other species (Fig. 12). A surprising result of our experiment was an overall extremely low recruitment that further declined as weevil density increased (Fig. 12). Coupled with a rather low overall impact on plant performance, we attribute some of the difficulties in showing a biologically significant impact of these stem miners to the design eliminating movement of adults. Plants in the field allow more numerous larval development of emergence of many more adults than were observed in our experiment and impact in the field was observed to be more dramatic than in our experiment.

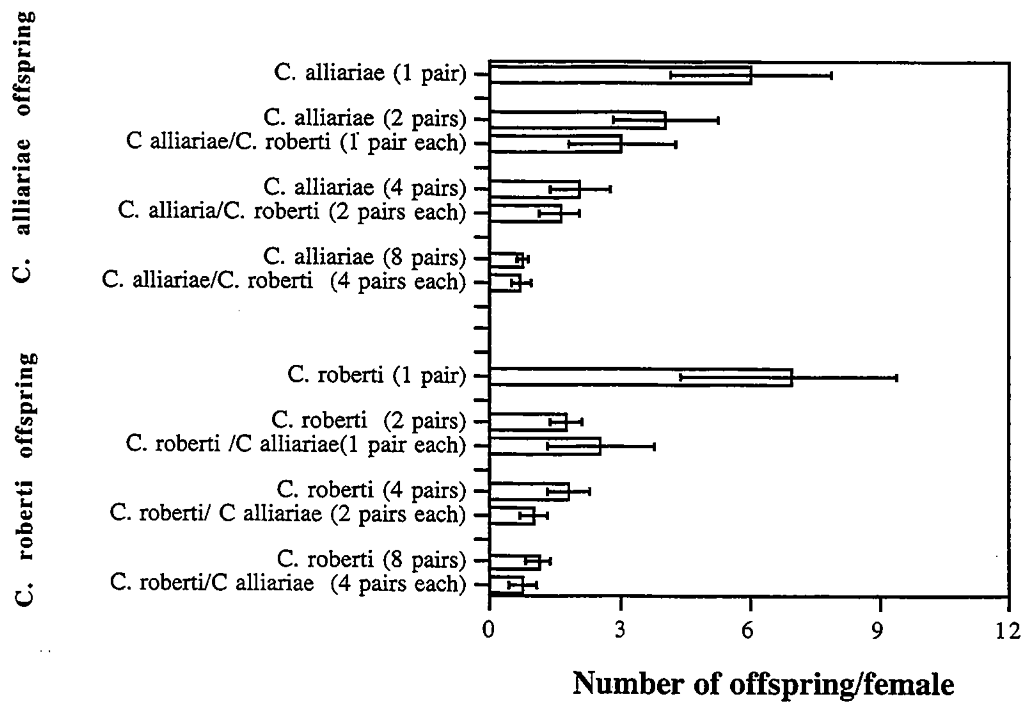

Fig. 12. Impact of weevil density (1, 2, 4 or 8 pairs) and weevil composition (C. alliaria alone, C. roberti alone, or mixed species pairs) on recruitment of *C. alliariae* and *C. roberti*. Data are means ±SE of 7-10 replicates/treatment.

Reasons for these "disappointing" results were discussed during visits to CABI in June and October 2000 (B. Blossey and V. Nuzzo). Rosettes and garlic mustard plants in the common garden in Switzerland had been kept under optimal conditions with an excess nutrient supply. Consequently they had grown to a size that was not observed in the field in North America or Europe. Plants may have had the ability to simply outgrow their natural enemies and compensate due to unlimited supply of resources (which would limit growth in the field). The need to cut back plants at the beginning of the growing season probably contributed to an increase in the number of shoots/plant. This in turn delayed the release of the control agents and "diluted" their impact (they are stem feeders).

We further investigated the potential mechanism for competition. Our hypothesis was that the decrease in offspring production observed with increasing weevil density in the competition/impact experiment was, at least in part, due to a reduction in the number of eggs laid per female. This result could reflect interference competition of females for limited oviposition sites. We therefore repeated an oviposition experiment with *C. alliariae* using some of the same densities as in the previous experiment, but reduced the length of infestation to avoid a shortage of oviposition sites. On 22 April 2002, 15 bolting plants of garlic mustard plants were randomly assigned to one of three treatments: 1, 2 or 4 pairs of *C. alliariae*. The plants were covered with gauze bags and weevils were released according to the different densities. After 2 days, on 24 April, weevils were removed and all plants dissected for eggs. One plant infested with one pair was not attacked and therefore excluded from the analysis. The number of eggs laid per female dramatically declined with increasing weevil density (Fig. 13). In some plants not all shoots were attacked, however, the number of unattacked shoots was independent of the density of weevils released onto plants ($F_{2,11} = 0.786$, $P = 0.480$). The result therefore suggests that interference competition of oviposition sites strongly limits the fecundity of *C. alliariae* at high densities. Most likely, similar results could be obtained with *C. roberti* although the species is more likely to lay larger egg clusters. This result is of critical importance in planned future releases or mass productions. High densities will not result in optimal recruitment and field releases in cages need to be planned accordingly.

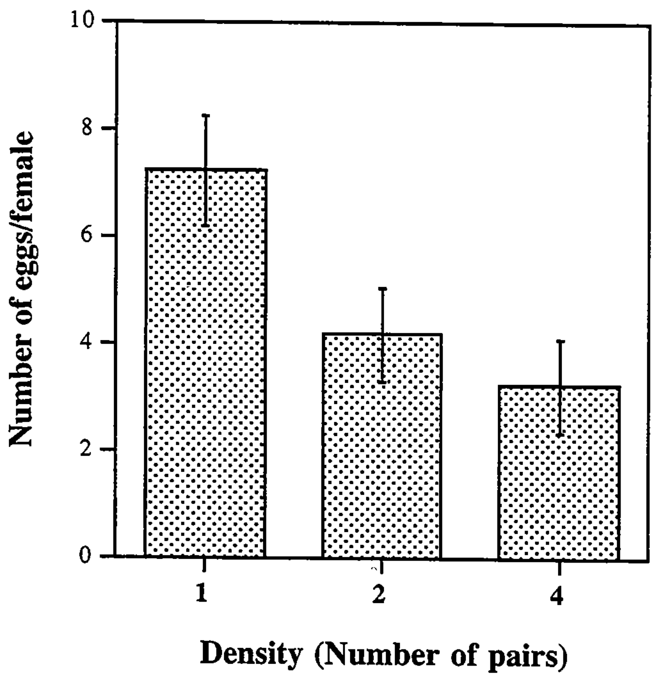

Fig. 13 Number of eggs laid by *C. alliariae* females kept at 3 different densities from 22-24 April 2002. Data are means (±SE) of five replicates each.

Natural enemies

Eliminating potential natural enemies is an essential requirement before shipping biological control agents into North America, therefore, eggs, larvae and adults of *C. alliariae* and *C. roberti* were field collected and held in cages, Petri dishes, or vials as appropriate to investigate parasitism of these various stages. Eggs laid singly could not be attributed to any species, eggs laid in clusters were attributed to *C. roberti*. We collected plants at three sites in the vicinity of Delémont, in the Rhine Valley and at Ticino between 9 April and 3 May 2002. All 252 eggs found in clusters and 56 eggs found singly upon stem dissections were retrieved and incubated in Petri dishes, and these checked daily for parasitoids and emerging larvae. A single egg-parasitoid emerged on 24 April 2002 from an egg cluster, so this was attributed to an attack on *C. roberti*. The species remains unidentified.

In cooperation with an ongoing project (see section on *C. constrictus*) at CABI targeting the Cabbage Seedpod Weevil, stem samples were dissected and the presence of parasitoids attacking larvae or pre-pupae of *C. roberti* or *C. alliariae* was recorded at field sites in Western Europe. Unattacked weevil larvae were kept in vials for pupation and all vials were kept over winter to allow for development and emergence of adult endoparasitoids. Parasitism rates ranged from 0 to 29% and were quite variable at different times at single collection sites (Table 2). Parasitoids were sent to a taxonomist for identification and all endoparasitoids reared from *C. alliariae*/*C. roberti* since 1999 were identified by Dr. Klaus Horstmann (University of Würzburg, Germany) as *Tersilochus obscurator* Aubert (Hymenoptera, Ichneumonidae), a solitary, koinobiontic endoparasitoid previously reported from *Ceutorhynchus pallidactylus*. We are awaiting identification of egg and larval ectoparasitoids which were sent to taxonomists for identification.

Several 100 adults of *C. roberti* and *C. alliariae* were checked for the presence of adult parasitoids in 2001 and again in 2002. We collected adults of both species on 27 February and 4 March 2002, i.e. presumably shortly after the end of overwintering at 3 sites around Delémont (site numbers 1, 6 and 10). Weevils were transferred into plastic cylinders ($n=4$) with a gauze bottom. A second cylinder was attached containing paper strips for pupation of emerging parasitoid larvae. Cylinders were regularly checked for cocoons of parasitoids. Adults were not attacked by parasitoids at any of the field sites investigated in 2001 and 2002, thus these two species may not have adult parasitoids and we may be able to ship them as adults to North America. This would greatly reduce the time they would need to spend in quarantine or in artificial rearing.

Table 3. Attack rates (%) of ecto- and endoparasites of *C. alliariae* (Berlin sites) and *C. alliariae* and *C. roberti* (all other sites) in 2001 and 2002.

Site	Collection date	Attack rates (%)		
		Ectoparasitoids	Endoparasitoids	Total
2001				
Delémont 4	30-May	11.5	1.8	13.3
Delémont 4	4-June	13.8	3.9	17.7
Delémont 4	11-June	15.2	0	15.2
Delémont 4	18-Jun	12.7	0	12.7
Delémont 4	26-June	29.3	0	29.3
Delémont 10	25 May	5.0	5.8	10.8
Delémont 33	5 June	3.9	0	3.9
Rhine Valley 1	21 May	4.0	22.2	26.2
Rhine Valley 1	31 May	14.4	8.1	22.5
Rhine Valley 1	6 June	12.7	0	12.7
Rhine Valley 1	13 June	9.8	0	9.8
Rhine Valley 1	19 June	18.3	0	18.3
Rhine Valley 2	21 May	0	17.8	17.8
Berlin 12	19 May	14.0	0	14
Berlin 18	20 May	0.4	1	1.4
Berlin 21	19 May	2.1	1.9	4.0
2002				
Berlin 15	9 June	0	0	0
Berlin 16	9 June	3.7	25	28.7

Ceutorhynchus scrobicollis

Life history

Ceutorhynchus scrobicollis is a root-mining weevil whose distribution is restricted to east and east central Europe. Adults emerge in May and June, consume leaves for a brief period, followed by summer aestivation. In Europe, oviposition begins in mid September and continues through the fall and spring (Fig. 14). Similar to the stem mining weevils *C. alliariae* and *C. roberti*, *C. scrobicollis* was found to be long-lived. Adults that emerged in early summer 2000 aestivated until September 2000, and completed their first oviposition period in early April 2001 (Fig. 14). Many of these adults were still alive at the end of the oviposition period, successfully aestivated in summer 2001, and completed a second oviposition period in fall/spring 2001/2002 (Fig. 14). Some weevils were still alive after their second oviposition period and aestivated in summer 2002. In October 2002, two pairs were found to be still alive and these were established for continued observation of oviposition as during the first two periods. Both females started producing eggs on 8 October 2002 and we are continuing to monitor their oviposition behavior. Both females were still alive in mid-December and had produced a similar number of eggs compared to the first two oviposition periods.

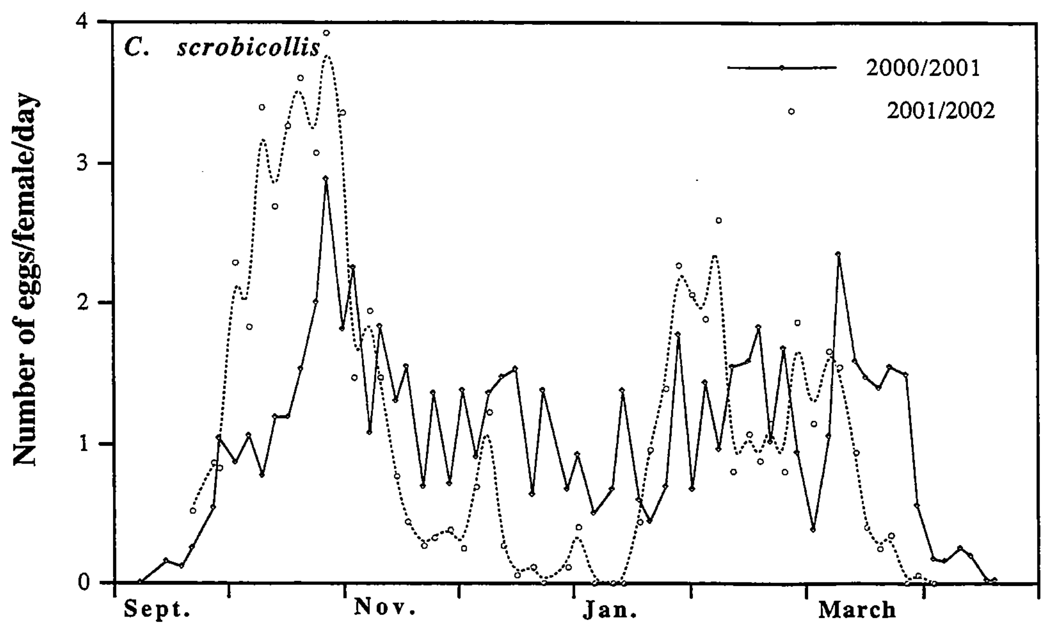

Fig. 14. Oviposition pattern of *C. scrobicollis* under ambient outside temperatures during fall/spring 2000/2001(diamonds) and 2001/2002(circles). A single pair was kept on cut shoots and foliage replaced every 3-5 days. Data are means±SE of 12 replicates (2000/2001) and 10 replicates (September-December) and 5 replicates (December-April) respectively (2001/2002).

The fecundity of *S. scrobicollis* over a single oviposition period (231.2±15.5 eggs and 243.6±26.4 eggs [mean ±SE of 12 females in year 1and 2, respectively]) is substantially higher than for the two stem mining weevils. Eggs were laid in about equal proportion in leaf petioles and into the leaf surface. However, hatch rates were only around 55% in either year, but this may be attributed to maintaining them in the laboratory where they hatched in 2-4 days instead of under ambient conditions outside. Temperatures in the outdoor shelter were recorded daily throughout the oviposition period of *C. scrobicolls* using a Hobo datalogger. The number of eggs laid per female/day was positively correlated with temperature with maximum egg production of 3-4 eggs at 15°C (Fig. 15). Surprisingly, females were able to continue their oviposition even if temperatures dropped below the freezing mark.

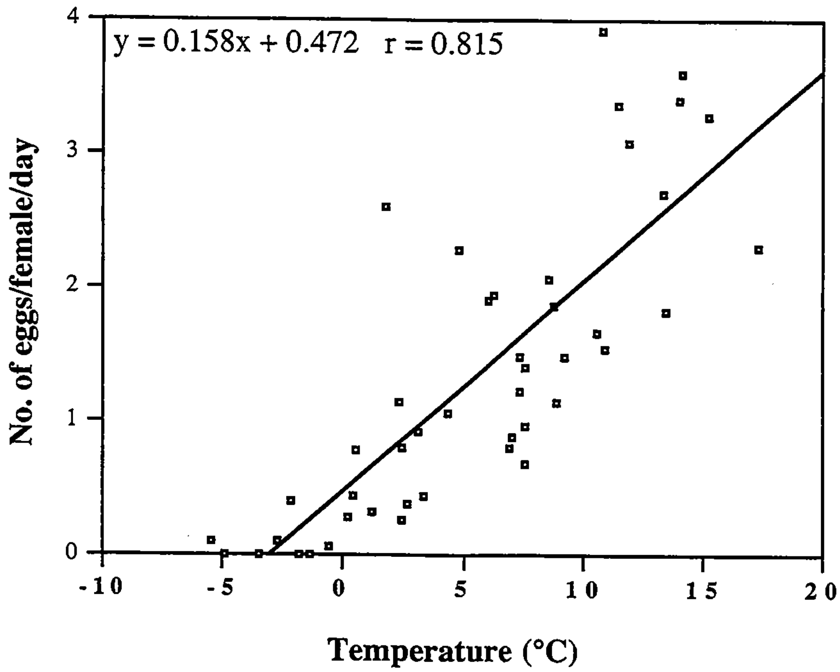

Fig. 15. Oviposition of *C. scrobicollis* (# of eggs/female/day) temperature (in 3-4 day intervals) during fall/spring 2001/2002.

Hatching larvae mine leaf veins and then continue downwards in petioles to growing points of rosettes. Second and third instars also feed in the cortex of root crowns and in the root. Larvae overwinter and continue feeding on garlic mustard plants and leave the host plant in early spring to pupate in the soil. The phenology of *C. scrobicollis* was investigated at a number of field sites in the vicinity of Berlin, Germany, in April and May 2001. Plants were excavated and stems and rosettes dissected and attack by *C. scrobicollis* and the respective life-stage was recorded (Fig. 16). Overall, a total of 1084 observations were made. In April the long oviposition period had resulted in asynchronous development of larvae; some larvae had already left the plants (indicated by the presence of exit holes) while females obviously were still actively ovipositing. By mid May, however, very few third instars remained while the presence of many exit holes was the only sign of attack during fall and spring (Fig. 16).

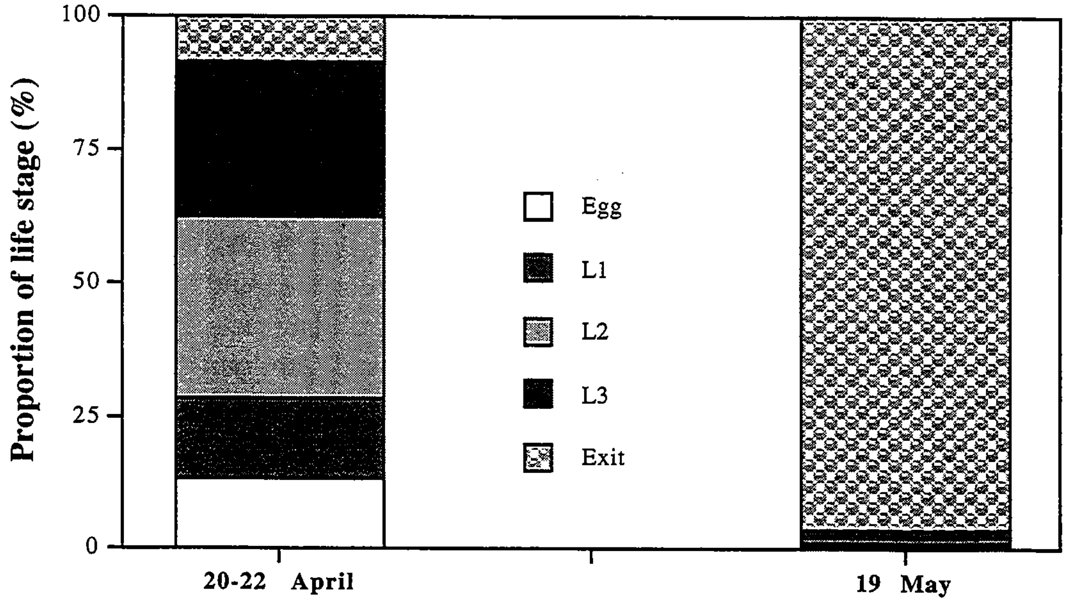

Fig. 16. Phenology of attack by various life stages of *C. scrobicollis* at several field sites around Berlin, Germany, in April and May 2001 (Exit represents exit holes in root or shoots of dissected plants). N=1084 observations.

Within the European distribution of *C. scrobicollis*, attack rates ranged from 4-96% of plants (Table 4), and usually several larvae complete development within a single plant. The maximum attack was 49 (egg-larvae) in a single plant. Attacked plants appeared water stressed, had reduced seed production and at high infestations, dried up prematurely (see impact experiments below).

Table 4. Attack rates (% of all plants) of *C. scrobicollis* at 5 field sites in the vicinity of Berlin, Germany, May 2001.

Site	# of plants dissected	Attack Rate (%)
Berlin 12	108	75
Berlin 25	23	61
Berlin 18	23	4
Berlin 21	99	70
Berlin 22	32	96

Impact of *C. scrobicollis* on performance of garlic mustard

Introduction

In fall 2000, we established an experiment to evaluate the impact of *C. scrobicollis* on plant performance and reproduction. This species is a fall breeder, i.e. adults aestivate in the summer, become active in September/October and then lay eggs into leaves and petioles of garlic mustard rosettes in the fall, winter and spring (Fig.14). Larvae feed in the petioles of rosette leaves and in the root-crown and leave their host plant in spring to pupate in the soil. We anticipate that attack of rosettes by *C. scrobicollis* will be of particular importance in the biocontrol program. Stressing rosettes should be more detrimental to plant performance than attack of the stems or of the seeds. To further investigate the shape of the relationship of herbivore attack and plant growth we established a common garden experiment at CABI varying the densities of attack by *C. scrobicollis*.

Methods

We germinated seeds on moist soil in spring 2000 and allowed seedlings and rosettes to establish in the absence of herbivory in gauze covered field cages. Adults for the experiment were obtained from either a rearing colony established at CABI or from field sites in the vicinity of Berlin, Germany. The details of the experiment are similar to those described for the impact competition experiment with *C. alliariae*/*C. roberti*. In fact, we used the same experimental site and cages developed for the stem feeders. All pots were established in the common garden and covered with gauze bags by the end of October 2000. Adults were released in different densities (0, 1, 2, 4 pairs) in October 2000 and allowed to oviposit for 4 weeks. To assess the effect of plant size on the impact of this species, we conducted the experiment using rosettes grouped into 2 different size classes (large and small). *C. scrobicollis* has an extended oviposition period spanning several months (Fig. 14), we therefore evaluated whether the time of oviposition (fall or spring) had any effect on the impact of this species by repeating the experiment in February 2001. Between 22 May and 4 July 2001, plants were regularly checked for emerging weevils and any discovered adults counted and removed. Pods that were already dry and ready to dehisce were removed and kept separately for each plant. On 22 May and 28 May the phenological stage of all plants was recorded and on 20 June, the height of each shoot measured. Between 4 and 31 July, the plants were harvested and the following parameters recorded: number of shoots per plant, height and base diameter of each shoot, number of dead and alive inflorescences per shoot, number of seed producing inflorescences per shoot, number of viable pods and seeds per plant. The plants were dissected and the number of dead and live larvae still present in the root, root crown or shoot was recorded and dry biomass of each plant measured.

Data analysis.

Plant parameters were analysed using MANOVA, because several dependent variables were measured. Weevil density, infestation period and plant size were entered as fixed factors. Subsequently, univariate ANOVA's were conducted with each dependent variable, and significance levels adjusted using a Bonferroni correction.

Results

The time of exposure/oviposition did not have any significant impact on any plant parameters and these results have been omitted for clarity in the following figures. Overall, only weevil density and plant size had significant impacts on plant performance.

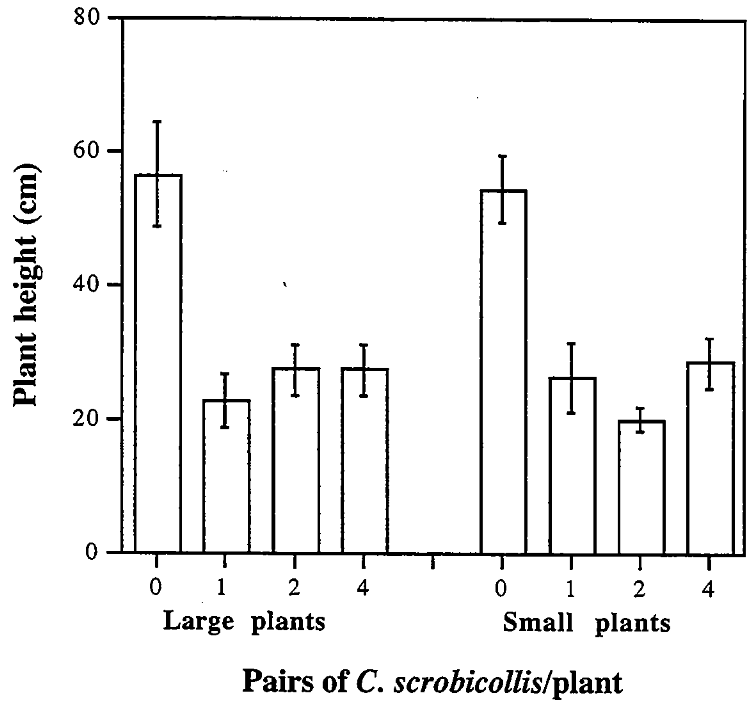

Fig. 17. Impact of plant size (large or small) and number of adult pairs of *C. scrobicollis* released/plant on plant height. Data are means±SE of 7-10 replicates/treatment

Smaller plants reached the same height as larger plants (Fig. 17) but under attack produced fewer shoots with smaller diameters (Fig. 18), fewer seed producing inflorescences (Fig. 19) and produced fewer seeds (Fig. 20) and produced less biomass (Fig. 21). Smaller plants had a significantly reduced recruitment of teneral adults (Fig. 22). With increased weevil density plants remained smaller (Fig. 17) although this decrease was already realized by attack from a single *C. scrobicollis* pair. With increased herbivore attack, plants produced more shoots (Fig. 18) with a decreased shoot base diameter (Fig. 18). The number of inflorescences was not affected by increasing weevil density (Fig. 19) while overall seed produced/plant (Fig. 20) and plant biomass (Fig. 21) declined but differences among treatments were not significant.

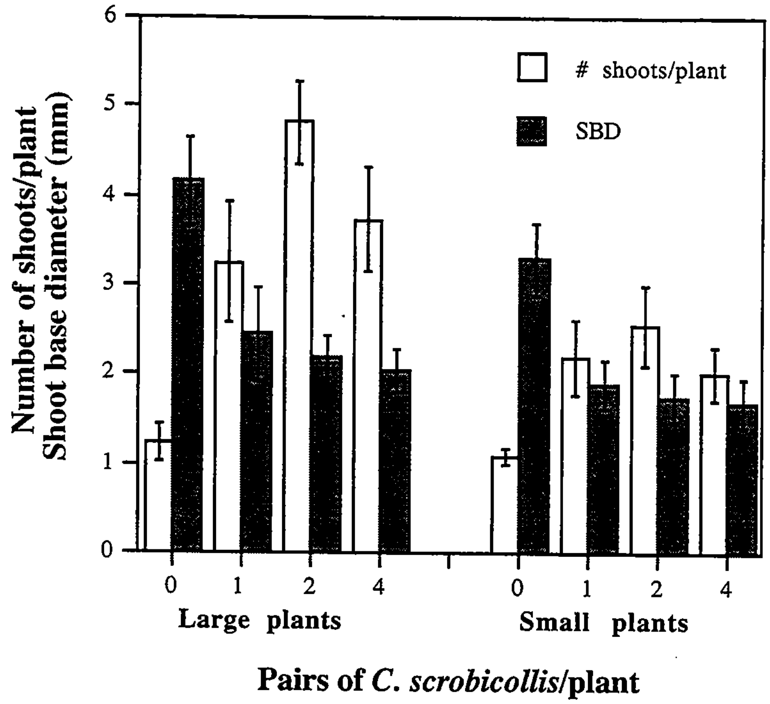

Fig. 18. Impact of plant size (large or small) and number of adult pairs of *C. scrobicollis* released/plant on number of shoots/plant (open columns) and mean shoot base diameter (in mm; SBD, filled columns). Data are means±SE of 7-10 replicates/treatment.

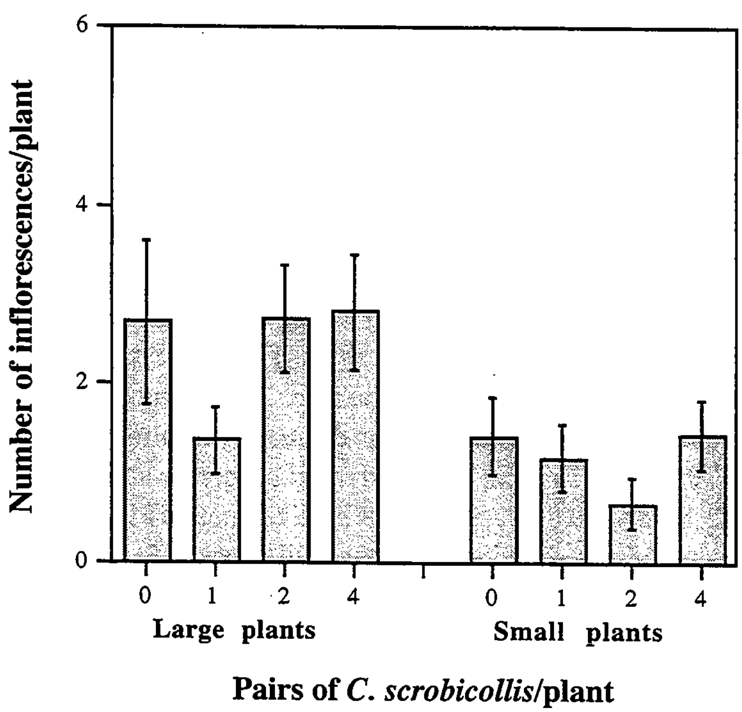

Fig. 19. Impact of plant size (large or small) and number of adult pairs of *C. scrobicollis* released/plant on number of inflorescences produced/plant. Data are means±SE of 7-10 replicates/treatment.

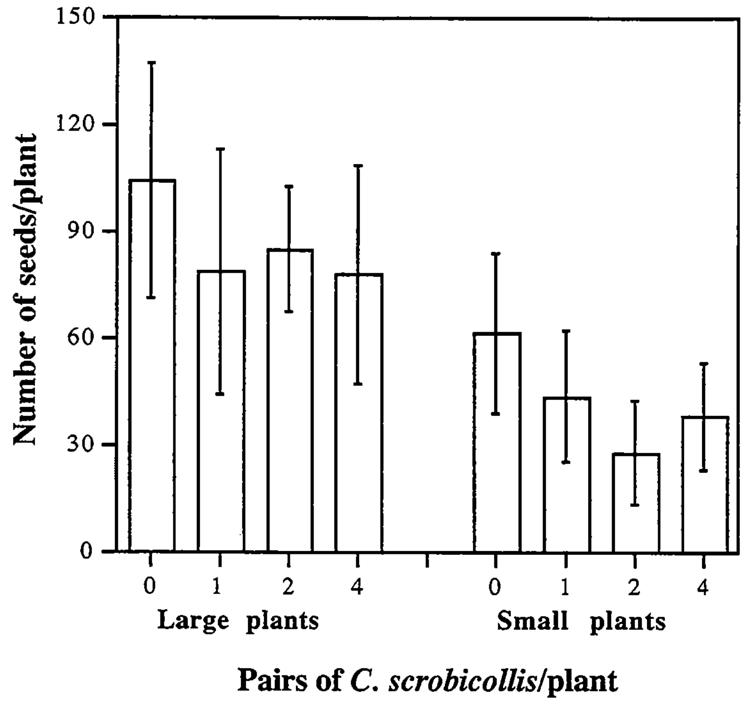

Fig. 20. Impact of plant size (large or small) and number of adult pairs of *C. scrobicollis* released/plant on number of seeds produced/plant. Data are means±SE of 7-10 replicates/treatment.

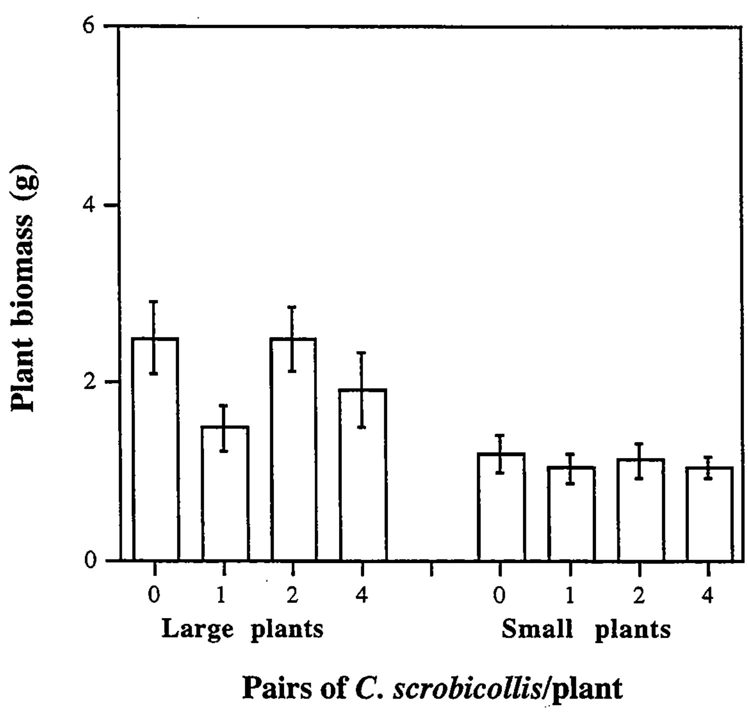

Fig. 21. Impact of plant size (large or small) and number of adult pairs of *C. scrobicollis* released/plant on dry plant biomass (g). Data are means±SE of 7-10 replicates/treatment.

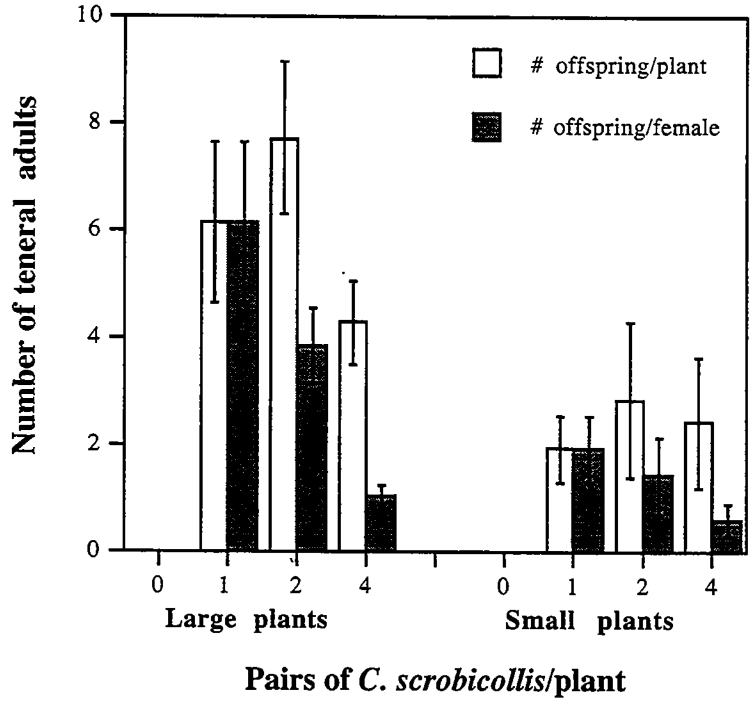

Fig. 22. Impact of plant size (large or small) and number of adult pairs of *C. scrobicollis* released/plant on recruitment (number of teneral adults) produced/plant (open columns) and per female (filled columns). Data are means±SE of 7-10 replicates/treatment.

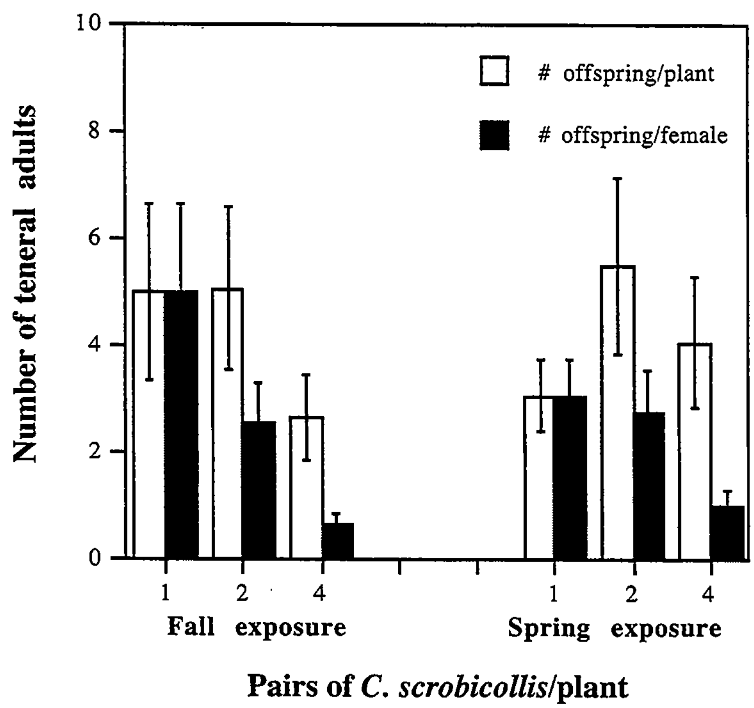

Fig. 23. Impact of exposure time (fall or spring) and number of adult pairs of *C. scrobicollis* released/plant on recruitment (number of teneral adults) produced/plant (open columns) and per female (filled columns).. Data are means±SE of 7-10 replicates/treatment.

Adult recruitment in our experiment was minimal, similar to experiments using the two stem-mining weevils (see above). This was most likely a result of significant intraspecific competition of a cohort of larvae hatching simultaneously. Increasing adult density resulted in a decrease in recruitment rather than the anticipated increase. We detected no difference in recruitment when attack was initiated either in the fall or in the spring (Fig. 23). Overall, for a biological control program, the effect of *C. scrobicollis* on plant performance appears more promising than that of the two stem feeders. Observations of plants attacked by *C. scrobicollis* in the field, and number of teneral adults emerging from individual plants are more promising than the results obtained in our impact studies. This is an indication of the limitations of experimental designs that necessarily restrict insect movement in impact studies. These are necessary shortcomings to allow experimental investigations while more sophisticated manipulations are either impossible or logistically difficult.

Competition between *C. scrobicollis* and a stem-mining weevil (*C. alliariae*)

Introduction

An important consideration for any introduction of multiple organisms in biological control programs is the potential for competitive interactions that may result in less successful control (Malecki et al. 1993, Masters et al. 1993, Blossey and Hunt-Joshi 2003). We evaluated the direct competitive interactions of the two stem mining weevils *C. roberti* and *C. alliariae* in a combined impact/competition experiment and concluded that intra and interspecific competition was only a function of weevil density, while weevil species identity did not matter. The two species were competitors with equal strength and neither species was superior. Therefore, we anticipate no interference in successful control of garlic mustard, except where plant resources limit the populations of the weevils. A particular concern in weed biocontrol programs is the potential for competition of spatially separated herbivores (for example above and below ground) mediated through their host plant.

The root-crown mining *C. scrobicollis* and the shoot miner *C. alliariae* generally attack garlic mustard plants at different times and occupy different spatial niches. While *C. alliariae* and *C. roberti* mainly oviposit into the shoots of bolting plants in spring, and larvae develop during spring and early summer, *C. scrobicollis* lays eggs from autumn until spring into the petioles and leaves of rosettes. Only during a short period in spring does temporal and spatial overlap of these species occur at field sites. Work by Masters, Brown and Gange (Gange and Brown 1989, Masters et al. 1993, Masters et al. 2001) has indicated that such competition may favor above-ground herbivores while below-ground herbivores suffer if their host plant is simultaneously attacked. While this appears of little consequence in the success of weed biocontrol programs (Blossey and Hunt-Joshi 2003), we wanted to evaluate the potential for such interactions with the weevil species considered as potential biological control agents of garlic mustard. Our impact experiment with C. *scrobicollis* has shown that attack can lead to a higher number of shoots, but with reductions in shoot base diameter and height (Fig.18). This in turn could affect the development and impact of *C. alliariae*. While an increase in the number of shoots may lead to an increase in oviposition sites for the stem mining weevils, a decrease in stem diameter and stem height may indicate reduced resource availability for larval development. Ideally, for improving the success of biocontrol for garlic mustard, we would prefer the impact of these two species be additive. Considering the possibility that both species may be introduced to North America, it is important to investigate potential negative interactions. We established an experiment to evaluate potential competitive interactions between *C. scrobicollis* and *C. alliariae*, representing a below- and an above-ground herbivore respectively.

Methods

We established plants in October 2000, by sowing garlic mustard seeds collected on 8 October 1999 in Lansing, NY USA. On 7 March 2001 seedlings were transferred individually into plug trays. On 18 June 2001, 200 rosettes were transplanted into plastic pots (14 cm diameter, 17 cm high) using a mixture of turf based garden soil (Florabella, 150-300 mg/l N), sand and vermiculite, and placed in a gauze-covered field cage (2 x 2 x 1.6 m) to protect against weevils occurring naturally in the Centre's garden. On 21 June 2001, rosettes were treated with insecticide (Confidor WG 70, Bayer, 0.02%) and on 10 July, against mites (Spomil Maag Agro, 0.15%). On 4 October 2001, 60 similar sized small plants and 60 similar sized large plants were selected and the number of leaves, length of longest leaf and diameter of largest leaf were recorded for each plant. Plant parameters were tested for potential pre-treatment differences. Using a full-factorial design, each plant was assigned to one of 18 treatments using (a) *C. scrobicollis* density (0, 1, and 2 pairs), (b) *C. alliariae* density (0, 1, and 2 pairs) and (c) plant size (large and small) resulting in 6 replicates per treatment.

Between 25 and 30 September 2001, *C. scrobicollis* collected in the area of Berlin/Halle, Germany, as well as adults reared at CABI were tested for their fecundity. One pair each was placed into a small transparent plastic cup (6.5 cm diameter, 7 cm high) covered with a gauze lid, and offered a cut petiole of garlic mustard inserted in a block of moist florist foam. After 2-3 days the petioles were dissected for eggs and only reproductive females were used in the experiment. On 4 October 2001, plants were individually covered with gauze bags, and weevils released according to treatments. Plants were searched for weevils and all adults were removed 25-27 October 2001. Plants were placed back into a common garden for overwintering. On 25 March 2002, all plants were dug into the ground about 50 cm apart and arranged in 6 rows (blocks) with each treatment occurring once (position determined at random) in each block. The first row was about 5m away from a forest edge, the last row about 10m. Plants were individually covered with gauze bags (55 cm diameter, 150 cm high) secured upright to a suspended wire running above each row. A wire ring (30 cm diameter) was fixed to the upper end of each gauze bag to prevent collapsing of the bag.

Adult *C. alliariae* were field collected and females individually tested for oviposition in small transparent plastic cups between 11 and 30 March 2002. Only ovipositing females were used in the experiment. All weevils were marked with white nail varnish on the elytra to allow separation of released individuals (which can be long-lived) and their offspring. Adults of *C. alliariae* were released on 30 March 2002 according to treatments.

On 25 March 2002, i.e. before release of *C. alliariae,* we measured the number and height of shoots for each plant. Starting 16 May (for plants infested with *C. scrobicollis*) and 26 June (for plants infested with *C. alliariae*), all plants were regularly searched for emerging weevils and their number and sex recorded. Seed pods that were dry and ready to dehisce were also harvested and kept separately for each plant. The phenology of all plants was recorded on 27 June, and all plants were harvested between 27 June and 19 August as soon as seed development was completed. We recorded: number of shoots per plant, height and base diameter of each shoot, number of inflorescences per shoot, and number of pods and viable seeds per plant. All plants were dissected and the number of dead and live *C. alliariae* larvae present in the shoot was recorded. To quantify attack of *C. alliariae,* each exit hole was counted as one larva that had already left the plant. Third instar larvae found alive were transferred into soil for pupation. In addition, the presence of attack by *C. scrobicollis* was verified during dissection.

Statistical analysis

Number of dead plants was analysed using the Multinomial Logistic Regression procedure in SPSS (version 10.0). In the subsequent analyses of plant parameters, only alive plants were included. Plant data were analysed with three-way ANOVAs using *C. scrobicollis* density (0, 1 and 2 pairs), *C. alliariae* density (0, 1 and 2 pairs) and plant size (large and small) as factors. Above-ground biomass and shoot base diameter were log10 transformed, and number of shoots and seeds square-root transformed to meet assumptions of ANOVA. Because multiple dependent variables were analyzed, the signifance level was adjusted using a Bonferroni correction, i.e. $P = 0.05/6 = 0.0083$. Insect data were analyzed separately for each species. Only plants onto which the respective insect species had been released were included in the analyses. Attack by *C. alliariae* was analysed by three-way ANOVAs, using *C. scrobicollis* density (0, 1 and 2 pairs), *C. alliariae* density (1 and 2 pairs), and rosette size (large and small) as factors. Because multiple dependent variables were analyzed, the significance level was adjusted using a Bonferroni correction, i.e. $P = 0.05/4 = 0.0125$. Because we did not anticipate an effect of *C. alliariae* on *C. scrobicollis,* the number of *C. scrobicollis* emerged was analyzed by a two-way ANOVA using *C. scrobicollis* density (1, and 2 pairs) and rosette size (large, small) as factors. The number of exit holes recorded and the number of adults emerging per plant or produced per female were square-root transformed to meet assumptions of ANOVA.

Results

The impact of the root feeder *C. scrobicollis* on plant performance was consistently greater than the impact of *C. alliariae*. Only attack by *C. scrobicollis* significantly reduced plant survival ($Chi^2 = 18.770$, d.f. = 2, $P < 0.001$) (Fig. 24), while rosette size and attack by *C. alliariae* had no influence on the number of plants that survived (Rosette size: $Chi^2 = 1.142$, df. = 1, $P = 0.285$; *C. alliariae:* $chi^2 = 0.1.800$, df. = 2, $P = 0.407$). Attack by *C. scrobicollis* reduced above ground biomass (Fig. 25), increased the number of shoots (Fig. 26), but decreased shoot base diameter (Fig. 27) and average plant height (Fig. 28), yet the effect on seed output was not significant (Fig. 29) (Table 5). While large rosettes produced on average two more shoots when attacked by *C. scrobicollis* compared to unattacked plants, the response of small rosettes was much weaker. Attack by *C. scrobicollis* often destroyed the central vegetation point, which broke apical dominance, and led to the production of 'secondary shoots' from dormant buds. The magnitude of this compensatory reaction depends on plant size. Shoots of plants attacked by *C. scrobicollis*, were thinner and shorter. Plants onto which two pairs had been released were on average 11 cm shorter than shoots of control plants. Biomass of these plants was reduced by 60%, and seed production by 48% compared to controls, although the latter effect was no longer significant after Bonferroni correction (Table 5).

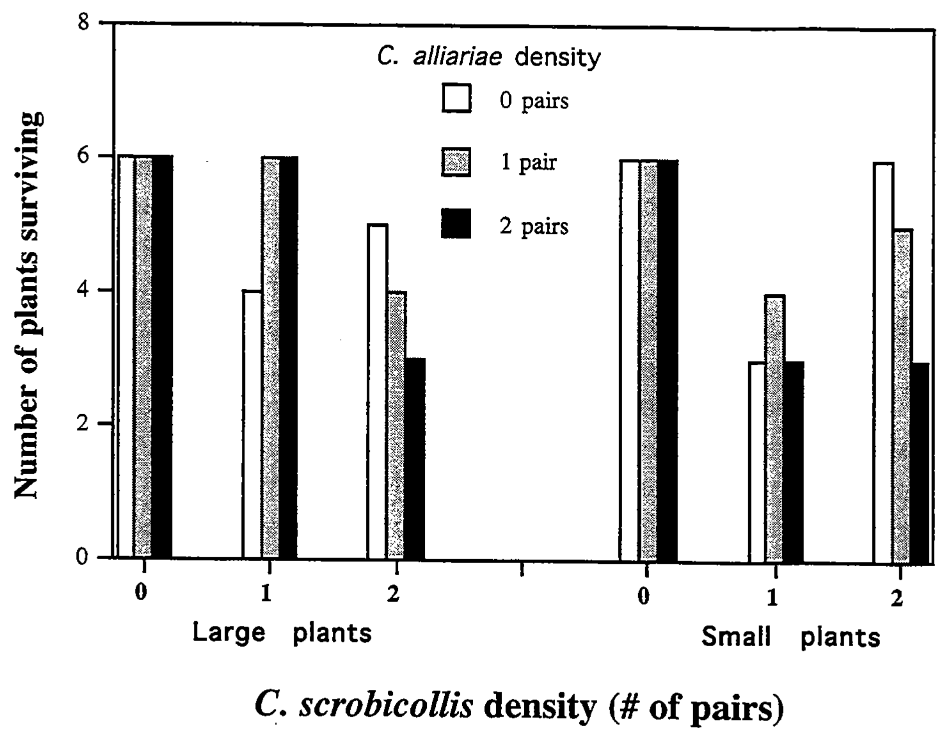

Fig.24. Survival of garlic mustard plants under different levels of herbivory by *C. scrobicollis* and *C. alliariae*. The experiment was started with six plants.

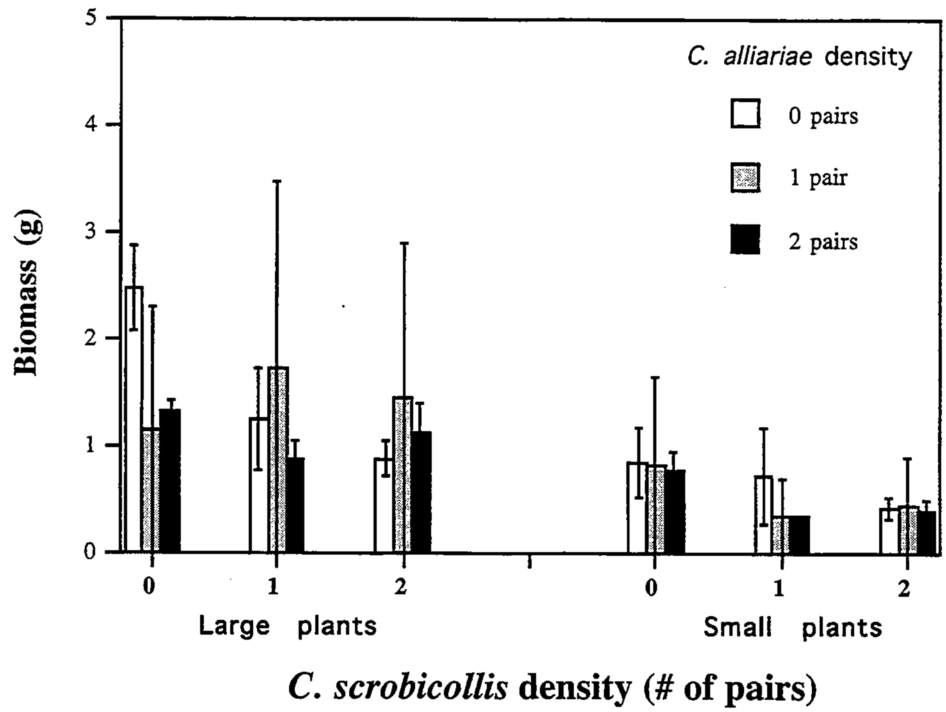

Fig.25. Above ground dry biomass (g) of garlic mustard plants under different levels of herbivory by *C. scrobicollis* and *C. alliariae*. Data are means ±SE of 6 replicates/treatment.

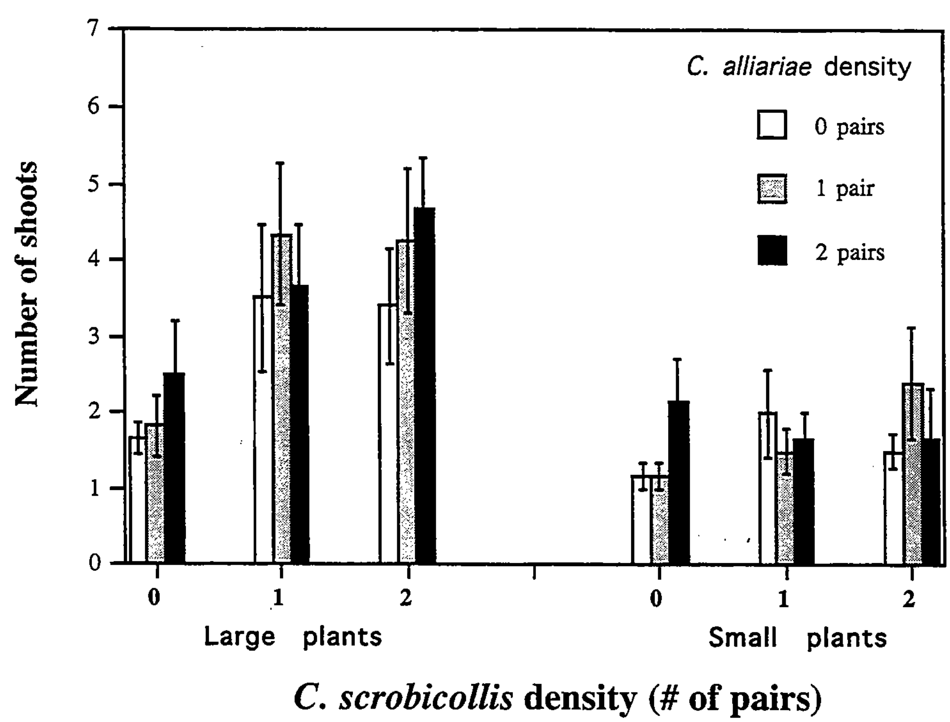

Fig.26. Number of shoots produced by garlic mustard plants under different levels of herbivory by *C. scrobicollis* and *C. alliariae*. Data are means ±SE of 6 replicates/treatment.

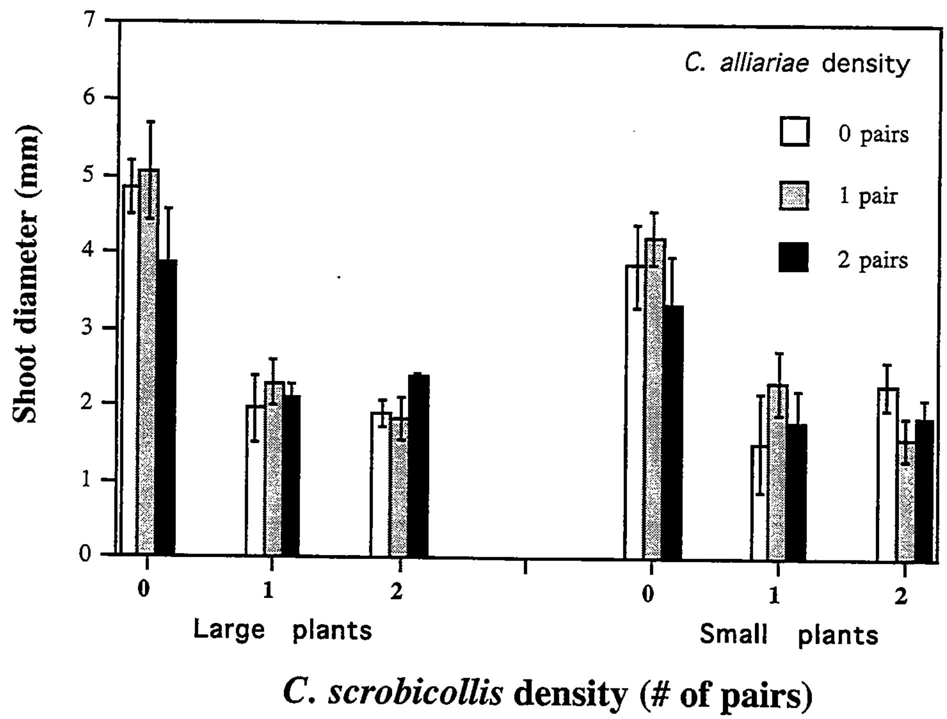

Fig.27. Basal shoot diameter (mm) of garlic mustard plants under different levels of herbivory by *C. scrobicollis* and *C. alliariae*. Data are means ±SE of 6 replicates/treatment.

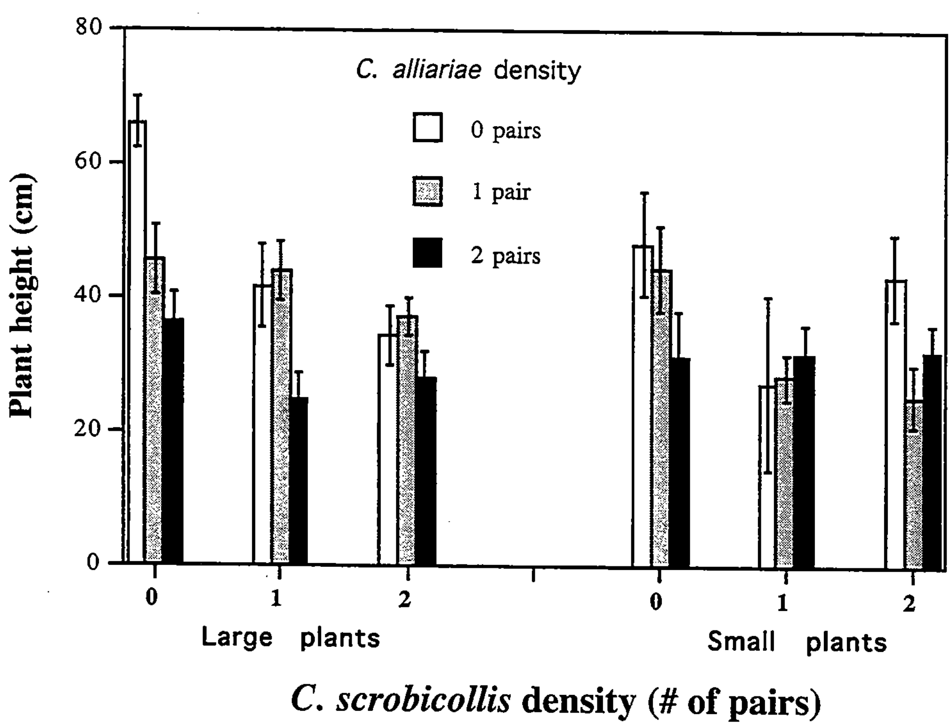

Fig.28. Plant height (cm) of garlic mustard plants under different levels of herbivory by *C. scrobicollis* and *C. alliariae*. Data are means ±SE of 6 replicates/treatment.

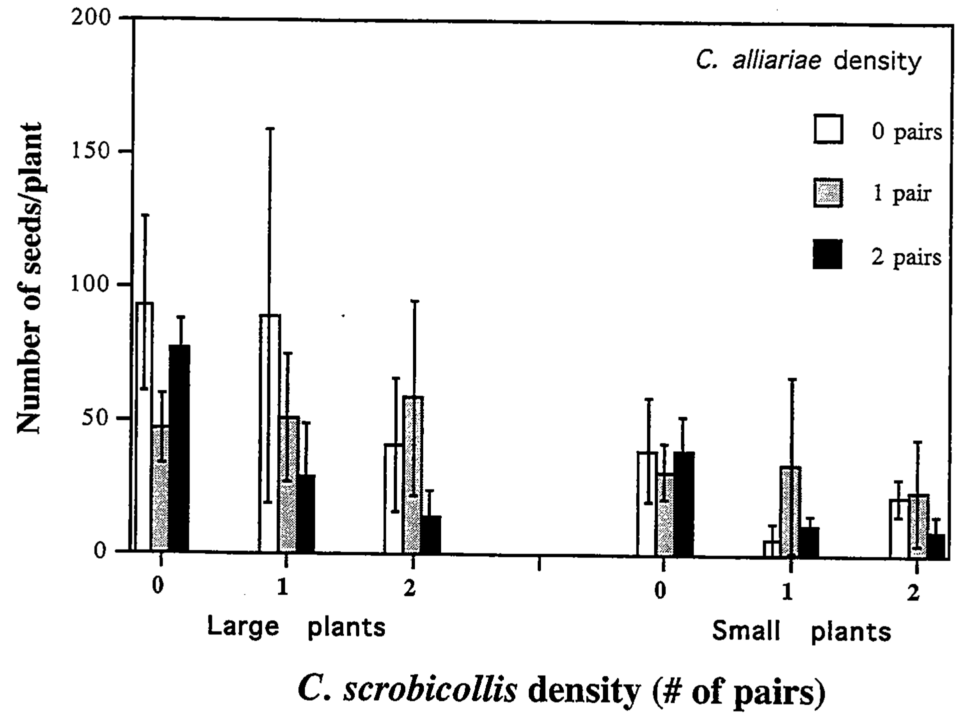

Fig.29. Seed production of garlic mustard plants under different levels of herbivory by *C. scrobicollis* and *C. alliariae*. Data are means ±SE of 6 replicates/treatment.

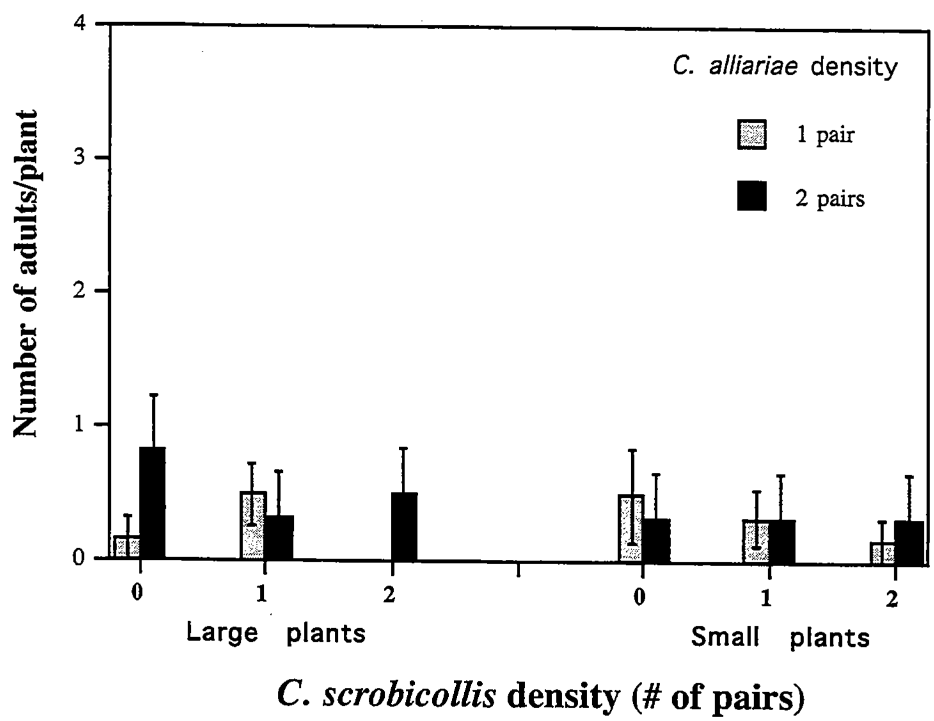

Fig. 30. Recruitment of *Ceutorhynchus alliariae* on garlic mustard plants under different levels of herbivory by *C. scrobicollis* and *C. alliariae*. Data are means ±SE of 6 replicates/treatment.

Table 5. Results of analysis of variance on the effect of *Ceutorhynchus scrobicollis* and *C. alliariae* at three different densities (0, 1, and 2 pairs) and initial plant size on plants of *Alliaria petiolata*. Because several dependent variables were analyzed, significance levels were adjusted using a Bonferroni correction (P = 0.05/6 = 0.0083). Therefore, only P-values < 0.0083 are considered significant.

Source of varaition		Above ground biomass		Number of shoots		Shoot height		Shoot base diameter		Number of inflorescences		Number of seeds	
	d.f	*F*	*P*	*F*	*P*	*P*	*F*	*F*	*P*	*F*	*P*	*F*	*P*
C. scrobicollis (Cs)	2	5.67	**0.005**	7.93	**0.001**	9.07	**<0.001**	45.08	**<0.001**	0.24	0.786	4.24	0.018
C. alliariae (Ca)	2	0.94	0.394	0.98	0.381	7.08	**0.002**	0.39	0.679	3.74	0.028	0.39	0.677
Plant size (S)	1	47.07	**<0.001**	30.12	**<0.001**	2.87	0.094	2.69	0.105	30.82	**<0.001**	7.24	0.009
Cs x S	2	0.15	0.858	3.41	0.038	1.14	0.326	0.45	0.641	0.01	0.993	0.26	0.773
Cs x Ca	4	1.44	0.229	0.81	0.523	1.41	0.238	2.23	0.074	0.71	0.590	0.32	0.866
Ca x S	2	0.43	0.649	0.15	0.862	1.26	0.291	0.05	0.953	0.13	0.879	0.28	0.757
Error	74												

Shoot height was the only plant variable significantly affected by attack of *C. alliariae*; shoots of plants onto which two weevil pairs had been released were on average about 15 cm shorter than those of control plants (Fig. 28, Table 5). Similar to the results of an earlier experiment, plants attacked by *C. alliariae* tended to produce more inflorescences. This change in plant architecture may be a response to larval mining. However, there was no impact on seed output (Fig. 29).

Our experiment produced results consistent with earlier data about the impact of root feeding and stem mining weevils on garlic mustard. The effect of the root-miner *C. scrobicollis* was generally stronger than the impact of the shoot-miner *C. alliariae*, when releasing the same number of adults. The interaction between the two species was not significant for any of the parameters investigated, suggesting that there is no negative competitive interaction. However, it also does not appear that at the weevil densities chosen for our experiment that *C. alliariae* contributes significantly to performance reductions of *A. petiolata*.

Insect data

All plants, except for three onto which only *C. alliariae* had been released, showed signs of larval mining, (and many of them had exit holes). Previous infestation of plants with *C. scrobicollis* reduced attack levels of *C. alliariae* (number of larvae and exit holes) at the individual shoot but not at the plant level. Attack by *C. scrobicollis* reduced shoot length and basal diameter, thereby reducing resource availability for the shoot-miner *C. alliariae.* Increasing the number of *C. alliariae* adults released/plant did not result in a higher attack level. This result confirms the earlier documented high intraspecific competition when confining weevils. Adult recruitment of *C. alliariae* was poor (Fig. 30); adults emerged from only half of the plants and in only very low numbers. Although in our experiments attack by *C. scrobicollis* did not negatively impact development of *C. alliariae*, field data where insects are allowed to interact may show different results. If both species were to be released in North America, a useful design would test the interaction of these species by releasing different combinations of insects at release sites and then follow the population build-up of both species.

Increasing weevil density of *C. scrobicollis* did not increase the number of F_1 weevils emerging per plant ($F_{1,63} = 0.026$, $P = 0.872$). The number of offspring per female was reduced when we placed more than a single female/plant ($F_{1,64} = 7.872$, $P = 0.007$; Fig. 31). We assume that the mechanism was, at least in part, resource limitation; adult recruitment was lower on small compared to larger rosettes ($F_{1,64} = 15.44$, $P < 0.001$).

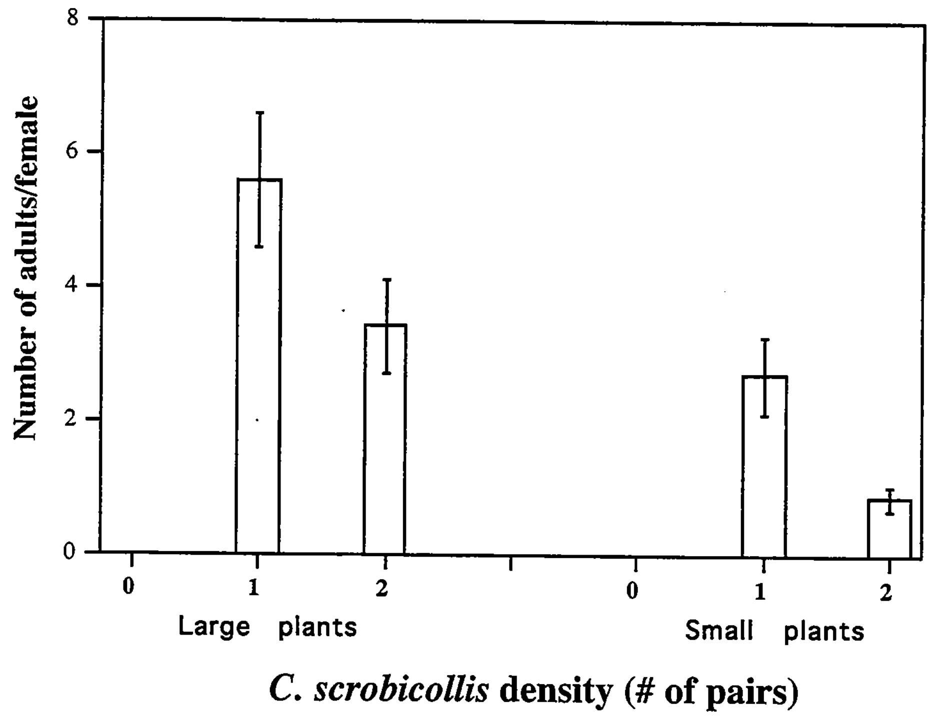

Fig. 30. Recruitment (number of teneral adults produced/female) of *Ceutorhynchus scrobicollis* on garlic mustard plants of different size under two different levels of herbivory. Data are means ±SE of 6 replicates/treatment.

Natural enemies

Larval samples taken in April and May at sites close to Berlin were investigated for presence of parasitoids. Parasitoid larvae attached to their host (ectoparasitoids) were transferred into Petri dishes lined with moist filter paper for pupation and adult emergence. Ectoparasitism of *C. scrobicollis* was low, and ranged between 3.2% and 4.2%. On 2 June 2001, 2 of the 6 incubated parasitoids emerged, and were sent to a taxonomist for identification. To investigate whether *C. scrobicollis* is attacked by adult parasitoids, weevils were collected at the same sites in April and May 2001. Parasitoid larvae emerged from only one site (Berlin 12), however, the attack rate was fairly high (20.5%). Between 22 and 25 May, adult parasitoids (Braconidae, Hymenoptera; probably *Microctonus* sp. syn *Perilitus*) emerged. The specimen were sent to a taxonomist for identification. The presence of adult parasitoids, even if only presently encountered at a single field site, will require additional safety precautions to avoid introduction of attacked adults to North America.

Phyllotreta ochripes

Life history

The flea beetle *Phyllotreta ochripes* attacks leaves (adults) and roots (larvae) of bolting *A. petiolata* plants as well as of rosettes. The species has at least a partial second generation and is probably multivoltine. *Phyllotreta ochripes* ranges widely over most of Europe and parts of northwestern Asia (Gruev and Döberl, 1997). Adults overwinter in the leaf litter and were found feeding on garlic mustard rosettes as early as the beginning of March. Females lay an average of 280 eggs from the end of April until the beginning of August. Eggs are laid into the soil close to root crowns and larvae usually mine just below the epidermis of roots or root crowns of bolting plants and rosettes. Mature larvae pupate in the soil and adults emerge by the end of June. Emergence of adults continues until the end of September. Development from first instar to adult takes 30 to 65 days.

Host specificity testing

The experimentally determined specificity of potential biocontrol agents for plants is **the** most important factor in determining whether USDA/APHIS will approve their importation and release. Strategies for developing a test plant list for experimental evaluation of potential biological control agents are based on a phylogenetic approach, where closely related species are considered to be of greater risk of attack than distantly related species (Wapshere 1974). In addition to phylogenetically related species, important agricultural plants, species growing in the same habitat as the target weed, related rare or endangered species and species with chemical similarities may be incorporated in the testing procedure. We developed such a list for garlic mustard comprising some 50 different plant species. (The full list of species is provided in Appendix A, including the reasons for inclusion in the test sequence). Over the past years the taxonomy of the Brassicaceae has been in flux and re-organizations have been proposed. As new literature and initial results were obtained, we included additional species in the testing sequence. For example, according to recent phylogenetic studies based on molecular analyses, *Thlaspi arvense*, *Peltaria alliaceae*, and *Sisymbrium irio* are very closely related to *A. petiolata* and were therefore included in tests conducted in 2002.

Rosettes, seeds, tubers, etc. of test plants were shipped to CABI, Switzerland where they were grown in a common garden and in a greenhouse until used for various tests. The differences in growing conditions caused problems with synchronization of a number of North American plants with insect activity that resulted in delays and need for additional plant material (see Discussion). During spring/summer 2000 we focused on testing the seed feeder *C. constrictus* and the flea beetle *P. ochripes* for their specificity. We carried out preliminary tests, - largely to test and improve procedures - for the other species under consideration. Host specificity tests were a major focus for the work during 2001 and 2002 and focused on the stem feeders and the root feeder.

In general, we employed a number of different tests: (1) sequential no-choice oviposition tests; (2) no-choice oviposition tests; (3) no-choice oviposition and larval development tests; and (4) multiple-choice oviposition and larval development tests. Depending on the particular test used, methods varied slightly; most tests were conducted using plant pieces offered to pairs of each tested insect for a determined period of time in the laboratory. Oviposition and development tests were usually conducted outside in a common garden using potted plants. Multiple-choice oviposition and development tests were conducted using large walk-in field cages. Details of the methods are described for each species where appropriate.

C. constrictus

Adult *C. constrictus* appear on their host when plants begin to flower. Females lay eggs into the developing seeds and larvae feed within the siliques. To assess oviposition and larval development, test plants need to be synchronized with the phenology of *C. constrictus*. In 2000 we tested oviposition of *C. constrictus* using 19 different plant species and continued to test additional species in 2001 using sequential no-choice tests. By the end of the 2001 field season, tests were completed with 36 different plant species. Tests continued during 2002 and by the end of the year a total of 45 species had been tested (Table 6).

Methods

Sequential no-choice tests

Two pairs or 2 females and a single male (slight differences between years) of *C. constrictus* were placed into a cage, and alternately offered cut shoots with ripening seeds of *A. petiolata* and ripening seeds of test plant species (=sequential no-choice test). Test plants were assigned randomly to each cage. For comparison (=control), pairs were offered *A. petiolata* only throughout the oviposition period. Every 2-3 days, plant material was exchanged, seeds dissected for eggs, the number of eggs recorded and the weevils provided with fresh material. Each exposure period was treated as one replicate. Replicates for test plants were only regarded as valid when test females continued to oviposit onto *A. petiolata* following a test. Cages were kept in a wooden hut at fluctuating temperatures.

Single-choice oviposition tests

Plant species accepted for oviposition in sequential no-choice tests were offered test plants under single-choice conditions. Cut shoots with ripening seeds of *Alliaria petiolata* and a test plant species were offered simultaneously to two pairs or 2 females plus a single male of *C. constrictus* kept in cylinders. Every 2-4 days, the plant material was exchanged and dissected for eggs, and weevils provided with fresh plant material. Test plants were assigned at random assuring that weevils in each cylinder were not offered any one test plant more than once. Each exposure period was treated as one replicate. Replicates were only considered valid if at least one egg was laid into the control or test plant. The number of replicates varied with test plant species (see Table 7).

No-choice oviposition and larval development tests

Tests varied slightly as improvements were made to the procedures. We report here the methodology used in 2002. Plant species accepted for oviposition in previous sequential no-choice tests and species considered critical were tested for their support of larval development. We also tested *Zea mays,* because it grows too tall to be tested in cylinders. Gauze bags were tied around seed bearing inflorescences of potted test or garlic mustard (control) plants, and two females and one male released for two weeks. Females were tested for fertility on cut garlic mustard shoots with developing seeds in cylinders as described above before being used in the tests. Only females that laid eggs were used in tests. Each inflorescence was regarded as one replicate, although more than one inflorescence was used for some plants. All inflorescences had at least three seedpods. Transparent plastic vials were attached to each bag with an elastic band and the inflorescences bent so that larvae leaving the pods would fall directly into the vials. Vials were checked for emerging larvae every morning starting two weeks after adults were removed from the gauze bags. Up to ten larvae were placed together in a vial filled two thirds with sifted soil for pupation. Vials were either kept in an underground insectary or in a fridge at constant 4°C for adult emergence in spring. In addition, all pods and seeds were dissected for signs of adult feeding, oviposition, and larval mining after emergence of larvae had ceased. Each time test plants were established, 2-4 plants of garlic mustard were set up as controls in the same way.

For *Zea mays* and *Armoracia lapathifolia*, two different test designs had to be used: Stems of *Zea mays* are very thick and could not be bent to retrieve mature larvae (see above). Therefore, whole plants were covered with gauze bags and weevils (2 females and 1 male) released onto each plant (n=4) on 24 May 2002. Two weeks later the weevils were removed and the surface of each pot covered with plaster. Plants were placed in screened cages (30 x 30 x 53 cm) arranged so that mature larvae leaving the pods would fall onto the plaster. The same method had been successfully used in 2001. The plaster was checked every morning for emerging larvae and these treated as described above.

Because *Armoracia lapathifolia* did not produce seeds at the Centre, seeding plants in a private garden were used instead. On 24 May 2002, seven inflorescences with seedpods were covered with gauze bags, and two females and one male were released in each bag. On 5 July, inflorescences were cut off the plant and brought back to the institute. Bags were searched for adults and larvae, and seedpods dissected for signs of adult feeding, oviposition, and larval mining.

Results

Sequential no-choice tests

Oviposition occurred on nine test species: *Armoracia lapathifolia, Arabis alpina, Barbarea vulgaris, Brassica nigra, Brassica napus, Brassica oleracea italica, Brassica rapa rapa, Rorippa amphibia and Raphanus sativus* (Table 6). The only species receiving a comparable number of eggs as the control, garlic mustard, was *A. lapathifolia*. However, eggs were laid directly into pods instead of into the seed. Seeds of *A. lapathifolia* are too small for *C. constrictus* oviposition, making it unlikely that larvae will develop. None of the test plants indigenous to North America were accepted for oviposition. The alternation of *A. petiolata* with test plant species reduced the overall fecundity of females, i.e. they laid fewer eggs compared to females provided continuously with *A. petiolata*. This is additional strong indication for the extreme specificity of this weevil.

Table 6. Summary of sequential no-choice tests for *Ceutorhynchus constrictus* .

Family Tribe *Species*	No. of replicates	No. of reps with eggs	Total no. eggs	Mean no. eggs/repl.
Brassicaceae				
Arabideae				
Alliaria petiolata (control)	**598**	**574**	**4425**	**7.4 ± 0.28**
Arabis alpina	5	2	4	0.8 ± 0.49
Arabis laevigata [a]	7	0	0	0
Armoracia lapathifolia = (A. rustica)	5	4	20	4.0 ± 2.02
Aubretia columnae	5	0	0	0
Barbarea vulgaris	7	2	7	1.0 ± 0.65
Cardamine bulbosa [a]	3	0	0	0
Nasturtium officinale	6	0	0	0
Rorippa amphibia	5	1	1	0.2 ± 0.2
Rorippa sylvestris	6	0	0	0
Sisymbrium irio	5	0	0	0
Alysseae				
Draba reptans [a]	5	0	0	0
Hesperis matronalis	5	0	0	0
Brassiceae				
Brassica napus napus	10	2	9	0.9 ± 0.64
Brassica nigra	13	4	6	0.46 ± 0.21
Brassica oleracea gemmifera	5	0	0	0
Brassica oleracea italica	5	1	1	0.2 ± 0.2
Brassica rapa rapa	5	1	1	0.2 ± 0.2
Raphanus sativus	5	3	5	1.0 ± 0.55
Sinapis alba	12	0	0	0
Lepidieae				
Cardaria draba	5	0	0	0
Camelina sativa	1	0	0	0
Capsella bursa-pastoris	5	0	0	0
Lepidium virginicum [a]	6	0	0	0
Thlaspi arvense	5	0	0	0
Resedaceae				
Reseda lutea	5	0	0	0
Poaceae				
Hystrix patula [a]	5	0	0	0
Triticum aestivum	5	0	0	0
Fabaceae				
Glycine max	6	0	0	0
Liliaceae				
Allium canadense [a]	1	0	0	0
Camasia scilloides	1	0	0	0
Trillium grandiflorum [a]	5	0	0	0
Smilacina racemosa [a]	8	0	0	0
Araceae				
Arisaema triphyllum [a]	5	0	0	0

Aristolochiaceae				
Asarum canadense [a]	6	0	0	0
Apiaceae				
Osmorhiza claytonii [a]	5	0	0	0
Rubiaceae				
Galium aparine [a]	8	0	0	0
Portulaceae				
Claytonia virginica [a]	5	0	0	0
Papaveraceae				
Sanguinaria canadensis [a]	4	0	0	0
Geraniaceae				
Geranium maculatum [a]	5	0	0	0
Boraginaceae				
Mertensia virginica [a]	5	0	0	0
Hydrophyllaceae				
Hydrophyllum virginicum [a]	7	0	0	0
Ranunculaceae				
Isopyrum biternatum [a]	6	0	0	0
Violaceae				
Viola sororia [a]	5	0	0	0
Polemoniaceae				
Phlox divaricata [a]	5	0	0	0

[a], Plant species indigenous to North America

Single choice oviposition tests
Only *Barbarea vulgaris* was accepted for oviposition under single-choice conditions (Table 7). However, the number of eggs laid into the test plant was much smaller than the number of eggs laid into garlic mustard. *Brassica nigra*, a species accepted under sequential no-choice conditions, was not attacked once beetles were given a choice.

Table 7. Results of single-choice oviposition tests conducted with *Ceutorhynchus constrictus*.

Tribe Species	No. of eggs laid on test/control plants					Total	% of eggs laid on test	 control	Factor of acceptance [a]
Arabideae									
Arabis alpina	0/8	0/6	0/12	0/13	0/4	0/43	0	100	0
Barbarea vulgaris	1/6	1/9	0/12	0/6	0/10	2/43	4	96	0.05
Rorippa amphibia	0/4	0/12	0/26	0/8	0/48	0/98	0	100	0
Brassiceae									
Brassica napus napus	0/10	0/14	0/15	0/26	0/10	0/75	0	100	0
Brassica nigra	0/10	0/8	0/13	0/7	0/4	0/42	0	100	0
Brassica oleracea italica	0/8	0/9	0/11	0/2	0/10	0/40	0	100	0
Brassica rapa rapa	0/7	0/12	0/11	0/1	0/2	0/33	0	100	0
Raphanus sativus	0/8	0/12	0/14	0/22	0/12	0/68	0	100	0

[a] control = 1

Larval development tests
All garlic mustard control plants were attacked, i.e. showed signs of larval feeding and/or larvae were found upon dissection or mature larvae were collected. Of the test plant species exposed, only *Brassica nigra* supported larval development (Table 8). But attack was much lower than on corresponding controls, and a large numberof dead larvae were found. Signs of adult feeding and oviposition were also found in three additional test plant species (*Arabis alpina, Rorippa amphibia, Brassica napus*), but no mature larvae emerged (Table 8). Unfortunately, the *A. lapathifolia* plants hardly produced any seeds and tests will be repeated to assure that this species is not a host for *C. constrictus*.

Because of conflicting results and attack of *C. constrictus* on *Brassica nigra* we established a single-choice development test with this species in 2002. One individually potted plant each of *B. nigra* and *A. petiolata* were placed so that one inflorescence of each plant could be covered together using a single gauze bag. We established 5 replicates, and two females and one male were released into each bag on 11 June 2002. On 21 June, the weevils were removed, and inflorescences of each plant species covered separately with a gauze bag. Mature larvae were collected and treated as described for the no-choice development tests. In addition, all pods and seeds were dissected for signs of mining. Mature larvae emerged from all five control plants, while pods of only two *B. nigra* plants showed signs of mining, and only three larvae emerged from one plant. This

Table 8. Summary of no-choice oviposition and development tests for *Ceutorhynchus constrictus* . Attacked seeds had feeding or oviposition holes or larval mining.

Family Tribe *Species*	No. repl.	No. repl. attacked	No. repl. with larval emergence	Total no. larvae	Mean larvae/ repl.
Brassicacea					
Arabideae					
Alliaria petiolata	**29**	**28**	**21**	**658**	**32.6 ± 7.1**
Arabis alpina	9	4	0	0	0.00
Armoracia lapathifolia	6	0	0	0	0.00
Barbarea vulgaris	5	0	0	0	0.00
Rorippa amphibia	5	5	0	0	0.00
Brassiceae					
Brassica napus napus	7	2	0	0	0.00
Brassica nigra	10	5	5	24	4.8 ± 2.6
Brassica oleracea gemmifera	3	0	0	0	0.00
Brassica oleracea italica	3	0	0	0	0.00
Brassica rapa rapa	4	2	0	0	0.00
Raphanus sativus	3	0	0	0	0.00
Poaceae					
Zea mays [a]	4	0	0	0	0.00

[a], plant species indigenous to North America

clearly shows that females of *C. constrictus* prefer garlic mustard under choice-conditions and that the attack we observe may be an artifact of the testing conditions. However, it is also clear that some larvae, albeit only a small percentage, have the ability to successfully complete development on *B. nigra*. We are planning to expose potted *B. nigra* and *A. petiolata* plants at a natural field site where *C. constrictus* occurs in 2003 to assess the potential for the species to be attacked under field conditions. We have not been able to test a number of plant species for this species due to difficulties in synchronizing insect and plant activity or lack of seed production. These tests will need to be completed during the 2003 field season or in quarantine in North America. We reserve discussion of these topics to the end of the host specificity testing part of this report.

C. roberti and C. alliariae

Preliminary results of sequential no-choice tests conducted in 2000 were not very encouraging due to the fact that females stopped oviposition after few exposures to non-host plants. However, tests conducted in 2001 and 2002 worked very well and we have made significant progress and a total of 48 plant species has been tested. For a number of species we are missing sufficient replication and we need to complete these tests during the next field season before a petition to TAG can be developed.

Methods

Sequential no-choice oviposition tests

We placed a single pair of *C. alliariae* or *C. roberti* into a transparent plastic cylinder (11 cm diameter, 15 cm high) with a gauze lid. Cut shoots or petioles of *A. petiolata* (control) and a test plant species were offered alternately. Cut plant parts were inserted in pieces of moist florist foam enclosed in plastic foil to keep them fresh. Test and control plants were offered alternately, i.e. test plants were placed in cylinders that previously contained *Alliaria*, and *Alliaria* plants were placed in cylinders that previously contained test plants. Test plants were randomly assigned to each cylinder assuring that weevil pairs were not offered any one test plant more than once. As comparison, 5 pairs of *C. alliariae* and 3 pairs of *C. roberti* were offered *A. petiolata* only throughout the oviposition period. After 2 - 3 days, plant material was exchanged and dissected for eggs. Each exposure period was treated as one replicate. Replicates were only regarded as valid when the female laid a minimum of one egg into the control that followed exposure to a test plant species.

Single-choice oviposition tests

Single-choice tests were conducted with test plant species and varieties accepted for oviposition under no-choice conditions in 2001 and 2002. One test plant species was offered simultaneously with the control, garlic mustard, to individual pairs in cylinders. After 3-4 days, the plant material was removed, dissected, the number of eggs recorded, and the weevils provided with fresh material. Test plants were randomly assigned to each cylinder assuring that individual weevil pairs were not exposed to any one test plant more than once. Replicates where only considered valid if a minimum of one egg was laid in the test or control plant.

No-choice oviposition and larval development tests

Test plant species accepted for oviposition in sequential no-choice tests and/or considered critical, were tested for their support of larval development. We released 2–3 females and 1-2 males onto gauze covered potted plants of garlic mustard (control) and of test species (see Table 9 for listing of species). In addition, all weevils released were marked with a spot of nail varnish on the elytra to be able to distinguish them from their potential offspring. The plants were kept outdoors in a common garden. After 2-4 weeks, plants were searched for released weevils, their number and sex recorded and signs of feeding or oviposition noted. After at least another 6 weeks, plants were searched for emerged weevils and dissected for signs of mining. Pots of all plants showing mining were kept under gauze and checked regularly until July for emerged weevils (Because not many individuals of the North American *Podophyllum peltatum* were available, we tested this species directly in larval development rather than in oviposition tests and plants were not dissected).

Results

Sequential no-choice oviposition tests

In general, females of *C. roberti* were more delicate/sensitive than females of *C. alliariae*, and more often stopped to lay eggs entirely after being offered a test plant. This was a consistent pattern observed in all years of our host specificity testing work and made obtaining valid replicates particularly difficult. However, this is a strong indication for the specificity of this species. Females of *C. alliariae* accepted 17 different test plant species (all in the family Brassicaceae) for oviposition, and females of *C. roberti* 11 (Table 9). Neither species laid eggs on any test plant species from other plant families. The average number of eggs laid was almost always higher for the control than for test plant species. *Draba reptans* and *Lepidium virginicum* were the only test plant species endemic to North America that were accepted for oviposition by *C. alliariae*; the latter species was also accepted by *C. roberti.*

Single-choice oviposition tests

For *C. alliariae* we were able to expose 17 plant species, and for *C. roberti,* 18 species in 1-5 replicates each (Tables 10, 11). Females of *C. alliariae* accepted 8 species for oviposition, including *Draba reptans,* a species indigenous to North America. Females of *C. roberti* accepted 10 of the species tested. Both weevils laid on average more eggs on the control than on the test plants, except that *C. roberti* actually preferred *Thlaspi arvense* under the testing conditions. However, we had to use thin, hard garlic mustard stems that may have no longer been very suitable for oviposition. In contrast, *T. arvense* plants had been kept in a greenhouse in order to protect them from attack by oligophagous insects occurring naturally in the Centre's garden. Therefore, their tissue was softer than that of garlic mustard, which might partly explain why they were so readily accepted for oviposition by *C. roberti.*

No-choice oviposition and larval development tests

In 2001, all garlic mustard control plants infested as references were attacked and adults emerged from five of these. For *C. alliariae*, no weevils emerged from any test plant species. Mining was found in three plants of *Brassica nigra*, two of *B. oleracea gemmifera* and in one of *B. rapa rapa*. However, the mines did not look typical for *C. alliariae* and might have been caused by polyphagous insects occurring naturally in the Centre's garden. From *B. nigra*, a weevil identified as *Ceutorhynchus pititarsis* (taxonomic confirmation pending) emerged. For *C. roberti*, only *Nasturtium officinalis* supported development of adults (Table 12). Three out of five *Nasturtium* plants showed mining and from all three, weevils emerged.

Table 9 Summary of sequential no choice tests for *Ceutorhynchus alliariae* and *C. roberti*.

Family / Tribe / Species	*C. alliariae*				*C. roberti*			
	No. repl.	No. repl. with eggs	Total no. eggs	Mean no. eggs/repl.	No. repl.	No. repl. with eggs	Total no. eggs	Mean no. eggs/repl.
Brassicaceae								
Arabideae								
Alliaria petiolata (control)	**593**	**520**	**1595**	**2.60 ± 0.09**	**302**	**271**	**1040**	**3.38 ± 0.14**
Arabis alpina	7	0	0	0	2	0	0	0
Armoracia lapathifolia = (A. rustica)	7	2	2	0.28 ± 0.18	5	0	0	0
Aubretia columnae	8	0	0	0	2	0	0	0
Barbarea vulgaris	5	0	0	0	3	0	0	0
Cardamine bulbosa a	5	2	4	0.8 ± 0.58	4	2	5	1.25 ± 0.75
Dentaria laciniata [a]	1	0	0	0	-	-	-	-
Nasturtium officinale	5	4	4	0.8 ± 0.2	4	2	3	0.75 ± 0.48
Rorippa amphibia	5	4	11	2.2 ± 0.86	3	1	1	0.33 ± 0.33
Rorippa sylvestris	5	0	0	0	-	-	-	-
Sisymbrium irio	5	3	4	0.8 ± 0.37	5	2	2	0.4 ± 0.24
Alysseae								
Draba reptans [a]	3	1	1	0.33 ± 0.33	1	0	0	0
Hesperis matronalis	5	0	0	0	5	0	0	0
Brassiceae								
Brassica napus napus	5	2	5	1.0 ± 0.63	5	1	1	0.2 ± 0.2
Brassica nigra	9	4	7	0.78 ± 0.36	5	2	5	1.0 ± 0.77
Brassica oleracea gemmifera	5	1	1	0.2 ± 0.2	1	0	0	0
Brassica oleracea italica	11	5	10	0.91 ± 0.39	6	1	2	0.33 ± 0.33
Brassica rapa rapa	5	4	5	1.0 ± 0.32	5	4	4	0.8 ± 0.2
Raphanus sativus	5	2	3	0.6 ± 0.4	2	0	0	0
Sinapis alba	9	3	3	0.33 ± 0.17	8	0	0	0
Lepidieae								
Cardaria draba	5	2	3	0.6 ± 0.4	-	-	-	-
Capsella bursa pastoris	8	0	0	0	6	0	0	0
Lepidium virginicum [a]	5	2	3	0.6 ± 0.4	1	1	4	4.0 ± 0
Thlaspi arvense	6	4	7	1.17 ± 0.60	5	4	13	2.6 ± 0.93
Peltaria alliacea	5	5	6	1.2 ± 0.2	3	2	3	1.0 ± 0.58
Resedaceae								
Reseda lutea	5	0	0	0	3	0	0	0
Poaceae								
Hystrix patula [a]	6	0	0	0	-	-	-	-
Triticum aestivum	5	0	0	0	3	0	0	0
Zea mays [a]	3	0	0	0	5	0	0	0
Fabaceae								
Glycine max	5	0	0	0	2	0	0	0
Liliaceae								
Allium canadense [a]	6	0	0	0	5	0	0	0
Trillium grandiflorum [a]	6	0	0	0	2	0	0	0
Smilacena racemosa [a]	6	0	0	0	1	0	0	0

Araceae								
Arisaema triphyllum [a]	6	0	0	0	-	-	-	-
Aristolochiaceae								
Asarum canadense [a]	11	0	0	0	5	0	0	0
Apiaceae								
Osmorhiza claytonii [a]	5	0	0	0	5	0	0	0
Rubiaceae								
Galium aparine [a]	5	0	0	0	-	-	-	-
Portulaceae								
Claytonia virginica [a]	5	0	0	0	3	0	0	0
Papaveraceae								
Sanguinaria canadensis [a]	5	0	0	0	8	0	0	0
Geraniaceae								
Geranium maculatum [a]	6	0	0	0	7	0	0	0
Boraginaceae								
Mertensia virginica [a]	12	0	0	0	11	0	0	0
Hydrophyllaceae								
Hydrophyllum virginianum [a]	5	0	0	0	8	0	0	0
Vitaceae								
Parthenocissus quinquefolia [a]	5	0	0	0	4	0	0	0
Asteraceae								
Solidago flexicaulis [a]	5	0	0	0	-	-	-	-
Ranunculaceae								
Isopyrum biternatum [a]	5	0	0	0	5	0	0	0
Violaceae								
Viola sororia [a]	6	0	0	0	5	0	0	0
Polemoniaceae								
Phlox divaricata [a]	7	0	0	0	5	0	0	0
Polygonaceae								
Polygonum virginicum [a]	5	0	0	0	-	-	-	-
Cannabaceae								
Cannabis sativa	-	-	-	-	3	0	0	0

[a], species indigenous to North America; ---, plant species not tested

Table 10. Results of single choice oviposition tests with *Ceutorhynchus alliariae.*

Family Tribe *Species*	Number of eggs laid on test/control plants						% of eggs laid on		Factor of acceptance (control = 1)
	Replicates					Total	Test	Control	
Brassicacea									
Alysseae									
Draba reptans [a]	2/2	0/1	0/4	0/5	0/3	2/15	12	88	0.13
Arabideae									
Armoracia lapathifolia	0/1	0/1	0/2	0/3	0/3	0/10	0	100	0
Dentaria laciniata [a]	0/3					0/3	0	100	0
Nasturtium officinale	0/1	0/2	0/2	0/2	0/1	0/8	0	100	0
Rorippa amphibia	0/5	2/0	8/3	3/0	0/12	13/20	39	61	0.65
Sisymbrium irio	1/1	1/0	0/5	0/6	1/3	3/15	17	83	0.20
Brassiceae									
Brassica napus napus	0/2	0/2	0/2	0/1	0/4	0/11	0	100	0
Brassica nigra	0/5	0/3	1/4	0/1	0/2	1/15	6	94	0.07
Brassica oleracea gemmifera	0/6	0/8	0/5	0/6	0/2	0/27	0	100	0
Brassica oleracea italica	0/5	0/8	0/5	0/4		0/22	0	100	0
Brassica rapa rapa	1/7	1/1	1/3	4/8	0/1	7/20	26	74	0.35
Raphanus sativus	1/1	0/3	0/2	1/2	2/1	4/9	31	69	0.44
Sinapis alba	0/3	3/0	0/1	0/1	0/1	3/5	38	62	0.60
Lepidieae									
Cardaria draba	0/4	0/8	0/4	0/8	0/13	0/37	0	100	0
Lepidium virginicum [a]	0/1	0/7	0/2	0/3	0/4	0/17	0	100	0
Thlaspi arvense	0/4	1/0	0/3	1/0	2/0	4/7	36	64	0.57
Peltaria alliacea	0/1	0/1	0/1	0/2	0/3	0/8	0	100	0

[a], plant species indigenous to North America

Table 11. Results of single-choice oviposition tests with *Ceutorhynchus roberti*.

Family / Tribe / *Species*	Number of eggs laid on test/control plants: Replicates					Total	% of eggs laid on: Test	Control	Factor of acceptance (control = 1)
Brassicacea									
Alysseae									
Draba reptans [a]	0/3	0/12	0/1	0/4	0/2	0/22	0	100	0
Arabideae									
Arabis alpina	0/2	0/2	0/4	0/6		0/14	0	100	0
Armoracia lapathifolia	0/6	0/4	0/10	0/2	0/4	0/26	0	100	0
Aubretia columnea	0/1	0/3				0/4	0	100	0
Barbarea vulgaris	0/7					0/7	0	100	0
Nasturtium officinale	8/0	0/5	0/2	0/4	1/5	9/16	36	64	0.56
Rorippa amphibia	0/1	1/1				1/2	33	67	0.50
Rorippa sylvestris	0/9	0/4	0/3	0/2	0/6	0/24	0	100	0
Sisymbrium irio	1/4	0/4	0/5	0/6	0/10	1/29	3	97	0.03
Brassiceae									
Brassica napus napus	2/3	4/6	2/0	0/2	2/3	10/14	42	58	0.71
Brassica nigra	0/6	0/9	3/5	0/6	0/4	3/30	9	91	0.10
Brassica oleracea italica	0/5	0/2	0/2	0/1	0/10	0/20	0	100	0
Brassica rapa rapa	0/4	0/2	5/7	0/11	0/3	5/27	16	84	0.19
Raphanus sativus	0/4	0/1	0/6	0/5	0/2	0/18	0	100	0
Sinapis alba	1/6	0/1	2/3	0/1	0/6	3/17	15	85	0.18
Lepidieae									
Lepidium virginicum [a]	1/2	0/1	0/2	0/3	0/6	1/14	7	93	0.07
Thlaspi arvense	5/1	4/0	3/2	1/0	5/5	18/8	69	31	2.25
Peltaria alliacea	0/1	0/3	1/5	0/2	0/1	1/12	8	92	0.08

[a], plant species indigenous to North America

Table 12. Summary of no-choice oviposition and development tests for *Ceutorhynchus alliariae* and *C. roberti.*

Family Tribe Species	*C. alliariae*					*C. roberti*				
	No. repl.	No. repli. with mining	No repl. with adult emergence	Total no. adults	Mean adults/ repl.	No. repl.	No. repli. with mining	No repl. with adult emergence	Total no. adults	Mean adults/ repl.
Brassicaceae										
Arabideae										
Alliaria petiolata	**22**	**22**	**8**	**27**	**3.4 ± 1.7**	**17**	**15**	**8**	**19**	**2.4 ± 1.0**
Armoracia lapathifolia	7	0	0	0	0	-	-	-	-	-
Nasturtium officinale	7	6	5	27	5.4 ± 1.5	5	3	3	11	3.7 ± 2.2
Rorippa amphibia	5	0	0	0	0	6	1	0	0	0
Sisymbrium irio	7	0	0	0	0	7	0	0	0	0
Alysseae										
Draba reptans [a]	7	0	0	0	0	7	0	0	0	0
Brassiceae										
Brassica napus napus	5	0	0	0	0	7	0	0	0	0
Brassica nigra	10	3	0	0	0	7	0	0	0	0
Brassica oleracea gemmifera	4	1	0	0	0	7	0	0	0	0
Brassica oleraceaa italica	7	0	0	0	0	7	0	0	0	0
Brassica rapa rapa	12	0	0	0	0	7	0	0	0	0
Raphanus sativus	7	0	0	0	0	-	-	-	-	-
Sinapis alba	3	0	0	0	0	13	0	0	0	0
Lepidieae										
Lepidium virginicum [a]	7	0	0	0	0	6	0	0	0	0
Thlaspi arvense	7	7	7	73	10.7 ± 2.0	7	3	2	16	8.0 ± 0
Berberidaceae										
Podophyllum peltatum [a]	7	0	0	0	0	-	-	-	-	-

[a], plant species indigenous to North America; ---, plant species not tested

The poor quality of our garlic mustard plants may have limited our ability to test more plant species in 2002. For *C. alliariae*, 12 replicates of four test plant species established were not considered valid, because no weevils emerged from control plants established at the same date. Adults emerged only from four control plants established with *C. alliariae* and eight established with *C. roberti,* however, nearly all control plants showed signs of mining. Both species, *C. alliariae* and *C. roberti* were able to complete development on two test plant species, *Nasturtium officinale* and *Thlaspi arvense* (Table 12). No sign of mining or adult emergence was found on any of the other species (including the indigenous *Draba reptans, Lepidium virginicum* and *Podophyllum peltatum*). On average more adults emerged from test than from control plants, another sign of the poor quality of the garlic mustard plants we needed to use in our tests.

Our tests indicate that both species are highly specific to garlic mustard. While we need to complete additional tests with plant species that were hard to grow or synchronize with our potential biocontrol agents, the two species that support larval development do not appear to be of particular concern. *Nasturtium officinale* is a plant of European origin that is thoroughly naturalized and sometimes considered invasive in North America. The species is listed under noxious weeds and invasive non-native plants - Eastern Region, USDA-Forest Service (http://www.fs.fed.us/r9/weed/nox-weed.htm). *Thlaspi arvense* is another European introduction to North America and considered a serious weed in grains and other crops. While we think that the poor quality of garlic mustard plants used in our tests may have contributed to the results, attack of either species in North America would not be of concern. In light of new taxonomic species arrangements and test results, we need to test more native North American species to exclude the possibility of non-target attack.

Ceutorhynchus scrobicollis

Adult *C. scrobicollis* reared at CABI or collected during field trips in October to Berlin were used for host-specificity tests established in autumn 2000. To verify that females were fertile, one pair was placed into a small transparent plastic cup (6.5 cm diameter, 7 cm high) covered with a gauze lid, and offered a cut leaf of garlic mustard inserted in a block of moist florist foam. After 2 to 6 days leaves were dissected for eggs. Only females that laid eggs were used for the tests.

Sequential no-choice oviposition tests
One pair of *C. scrobicollis* was placed in a transparent plastic cylinder (11 cm diameter, 15 cm tall) with a gauze lid. Weevils were alternately offered cut petioles of *A. petiolata* (control) or a test plant species. After 2-3 days, plant material was removed, checked for feeding marks, dissected, the number of eggs recorded, and new plant material placed in the cylinders. Test plants were randomly assigned to each cylinder assuring that weevil pairs were offered each test plant no more than once. Each exposure period was treated as one replicate. Replicates in which no oviposition occurred on test plants were considered valid only if the females laid a minimum of one egg into the control that followed. Cylinders were kept in a wooden hut at ambient temperatures.

Single-choice oviposition tests
Plant species and varieties of *Brassica oleracea* accepted for oviposition under no-choice conditions were offered simultaneously with garlic mustard to pairs of *C. scrobicollis* kept in cylinders. Seven plant species and 5 varieties of *Brassica oleracea* were tested, (3–5 replicates each) between 29 September and 1 November 2001 and additional species were tested during the 2002 field season (Table 13). Cut petioles of the test plant were offered with petioles of garlic mustard in a moist florist foam. After 3-4 days, plant material was removed and dissected for eggs. Test plants were randomly assigned to each cylinder but individual weevil pairs were not exposed to any test plant more than once. Replicates were only regarded as valid when a minimum of one egg was laid in the test or control plant.

No-choice oviposition and larval development tests
On 28 October 2001, no-choice development tests were established with ten plant species that had been accepted for oviposition in previous tests. All plants were overwintered in a garden bed at the institute. Between 15 May and 3 July 2002, plants were regularly examined for emerging weevils, and their number and sex recorded. On 3 July, plants were dissected and examined for signs of larval mining. North American test plants and *Armoracia lapathifolia* were not dissected to save plant material. On 18 October 2002, additional development tests were established with *Arabis alpina*, *Brassica oleracea capitata sabauda* "Wirz Vorbote 3", *Peltaria alliacea* and *Rorippa sylvestris*. All plants had been kept in cages or in a greenhouse prior to tests to prevent contamination by feral oligophagous herbivores.

Table 13. Summary of sequential no choice tests for *Ceutorhynchus scrobicollis.*

Family Tribe Species	No. repl.	No. repl. with eggs	Total no. eggs	Mean no. eggs/repl.
Brassicaceae				
Arabideae				
Alliaria petiolata (control)	**684**	**634**	**2916**	**4.26 ± 1.3**
Arabis alpina	10	2	2	0.2 ± 0.13
Arabis laevigata [a]	10	4	13	1.3 ± 0.65
Armoracia lapathifolia = (A. rustica)	12	1	4	0.33 ± 0.33
Aubretia columnae	10	0	0	0
Barbarea vulgaris	10	2	7	0.70 ± 0.60
Cardamine bulbosa [a]	2	0	0	0
Dentaria heterophylla	1	1	1	1.0 ± 1.0
Dentaria laciniata [a]	11	3	4	0.36 ± 0.20
Nasturtium officinale	10	4	8	0.80 ± 0.49
Rorippa amphibia	10	6	20	2.0 ± 1.05
Rorippa sylvestris	12	1	1	0.08 ± 0.08
Sisymbrium irio	10	0	0	0
Alysseae				
Draba reptans [a]	10	7	10	1.0 ± 0.26
Hesperis matronalis	10	0	0	0
Brassiceae				
Brassica napus napus	10	4	6	0.60 ± 0.27
Brassica nigra	10	3	4	0.40 ± 0.22
Brassica oleracea gemmifera	10	0	0	0
Brassica oleracea italica	10	0	0	0
Brassica oleracea rubra "Ruby perfection"	10	2	5	0.5 ± 0.34
Brassica oleracea sabauda "Eiskönig"	11	0	0	0
Brassica oleracea sabauda "Paradisler"	15	3	13	0.86 ± 0.58
Brassica oleracea sebellica	11	3	4	0.36 ± 0.2
Brassica oleracea gonglyodes "Cindy"	10	1	1	0.1 ± 0.1
Brassica oleracea capitata sabauda	11	2	4	0.36 ± 0.28
Brassica rapa rapa	10	4	15	1.50 ± 0.70
Cakile edentula	12	11	39	3.25 ± 0.72
Raphanus sativus	10	2	2	0.2 ± 0.13
Sinapis alba	11	3	5	0.45 ± 0.28
Lepidieae				
Cardaria draba	10	4	9	0.9 ± 0.5
Capsella bursa pastoris	10	0	0	0
Lepidium virginicum [a]	10	5	9	0.9 ± 0.38
Thlaspi arvense	10	10	47	4.70 ± 0.86
Peltaria alliacea	10	10	81	8.10 ± 0.78
Trapaeolaceae				
Trapaeolum majus	10	0	0	0
Resedaceae				
Reseda lutea	10	0	0	0

Poaceae				
Hystrix patula a	3	0	0	0
Triticum aestivum	10	0	0	0
Zea mays [a]	10	0	0	0
Fabaceae				
Glycine max	10	0	0	0
Liliaceae				
Allium canadense [a]	10	0	0	0
Aristolochiaceae				
Asarum canadense [a]	17	0	0	0
Apiaceae				
Osmorhiza claytonii [a]	10	0	0	0
Rubiaceae				
Galium aparine [a]	10	0	0	0
Portulaceae				
Claytonia virginica [a]	10	0	0	0
Papaveraceae				
Sanguinaria canadensis [a]	8	0	0	0
Geraniaceae				
Geranium maculatum [a]	10	0	0	0
Boraginaceae				
Mertensia virginica [a]	8	0	0	0
Hydrophyllaceae				
Hydrophyllum virginianum [a]	12	1	1*	0.08 ± 0.08
Berberidae				
Podophyllum peltatum [a]	2	0	0	0
Vitaceae				
Parthenocissus quinquefolia [a]	2	0	0	0
Asteraceae				
Solidago flexicaulis [a]	10	0	0	0
Ranunculaceae				
Isopyrum biternatum [a]	13	0	0	0
Violaceae				
Viola sororia [a]	10	0	1*	0.09 ± 0.10
Polemoniaceae				
Phlox divaricata [a]	10	0	1*	0.09 ± 0.10

[a], species native to North America; *, egg laid on the outside of the leaf.

Table 14. Summary of single-choice oviposition tests for *Ceutorhynchus scrobicollis* .

Family / Tribe / Species	Number of eggs laid on test/control plants: Replicates 1	2	3	4	5	6	7	8	9	10	11	Total	% of eggs on Test	% of eggs on Control	Factor of acceptance (control = 1)
Brassicaceae															
Alysseae															
Draba reptans [a]	0/6	1/19	0/7	1/5	0/5							2/42	4.55	95.45	0.05
Arabideae															
Arabis alpina	0/11	0/7	0/3	0/5	0/7							0/33	0	100	0
Armoracia lapathifolia (A. rustica)	1/2	0/3	6/2	0/10	0/9							7/26	21	79	0.27
Barbarea vulgaris	2/13	1/13	3/2	0/6	4/2							10/36	22	78	0.28
Nasturtium officinale	2/19	2/11	0/6	0/8	1/7							5/51	8.93	91.07	0.10
Rorippa amphibia	0/9	0/15	0/1	0/1	0/2	1/1	1/4	0/10	2/4	1/11	0/12	5/70	7	93	0.07
Rorippa sylvestris	2/2	0/4	0/3	0/4	0/6							2/19	9.52	90.48	0.11
Brassiceae															
Cakile edentula [a]	6/8	7/5	0/5	4/8	2/6							19/32	37.25	62.75	0.59
Brassica napus napus	0/7	0/8	0/4	0/5	0/5							0/29	0	100	0
Brassica nigra	3/3	7/4	3/6	3/8	2/5							18/26	40.91	59.09	0.69
Brassica oleracea rubra "Ruby perfection"	0/3	0/2	0/19	0/19	0/18							0/61	0	100	0
Brassica oleracea sabauda "Eiskoenig"	0/7	0/6	0/8	0/7	8/10							8/38	17	83	0.21
Brassica oleracea sabauda "Paradisler"	0/10	0/13	0/2	0/11	0/3	0/4						0/43	0	100	0
Brassica oleracea sebellica	0/6	0/15	0/13	0/2	2/21							2/57	3	97	0.04
Brassica oleracea gonglyodes "Cindy"	0/7	0/8	0/19	0/18	0/14							0/66	0	100	0
Brassica oleracea capitata sabauda	0/10	0/9	1/3	0/7	1/5							2/34	5.56	94.44	0.06
Brassica rapa rapa	0/20	0/17	3/4	1/11	9/8							13/60	17.81	82.19	0.22
Raphanus sativus	0/9	0/14	0/10	0/6	0/4							0/43	0	100	0
Sinapis alba	0/8	0/7	3/3	7/0	1/7							11/25	30.56	69.44	0.44
Lepidieae															
Cardaria draba	0/1	0/2	0/5	0/3	1/10							1/21	5	95	0.05
Lepidium virginicum [a]	1/3	0/7	0/6	0/5	0/6							1/27	3.57	96.43	0.04
Peltaria alliacea	5/4	3/6	3/5	4/5	7/4							22/24	47.83	52.17	0.92
Thlaspi arvense	4/3	8/0	14/6	9/1	6/3							41/13	75.93	24.07	3.15
Hydrophyllaceae															
Hydrophyllum virginianum [a]	0/2	0/5	0/4	0/12	0/10							0/33	0	100	0
Violaceae															
Viola sororia [a]	0/6	0/13	0/23	0/6	0/15							0/63	0	100	0
Polemoniaceae															
Phlox divaricata [a]	0/9	0/10	0/6	0/14	0/6							0/45	0	100	0

[a], species native to North America

Table 15. Summary of no-choice ovipostion and development tests for *Ceutorhynchus scrobicollis* .

Family Tribe *Species*	No. repl.	No. repl. with mining	No. repl. with adult emergence	Total no. adults	Mean adults/ repl.
Brassicaceae					
Arabideae					
Alliaria petiolata	**24**	**23**	**22**	**243**	**11.0 ± 2.1**
Arabis laevigata [a]	7	0	2	2	1*
Armoracia lapathifolia	10	0	2	3	1.5*
Barbarea vulgaris	10	1	2	2	1*
Rorippa amphibia	10	0	1	1	1*
Alysseae					
Hesperis matronalis	5	0	0	0	0
Brassiceae					
Brassica napus var. napus	5	2	1	1	1*
Brassica nigra	9	4	0	0	0
Brassica oleracea italica	5	0	0	0	0
Brassica oleracea gemmifera	5	0	0	0	0
Brassica oleracea rubra "Ruby perfection"	5	0	0	0	0
Brassica oleracea sabauda	5	1	1	1	1
Brassica oleracea sabauda " Paradisler"	5	0	0	0	0
Brassica oleracea sebellica	5	0	0	0	0
Brassica oleracea souglyodes "Cindy"	5	0	0	0	0
Lepidieae					
Capsella bursa-pastoris	5	0	0	0	0
Resedaceae					
Reseda lutea	5	0	2	2	1*
Hydrophyllaceae					
Hydrophyllum virginianum [a]	5	0	0	0	0
Violaceae					
Viola sororia [a]	5	0	0	0	0
Polemoniaceae					
Phlox divaricata [a]	5	0	0	0	0

[a], plant species indigenous to North America

*, Adults are most likely unrecovered weevils that were released onto plants for oviposition because no mining was detected upon dissection.

Two females and one male of *C. scrobicollis* were released onto potted, gauze-covered rosettes of garlic mustard (control) or test species. Seven replicates were established per species. To verify that females were fertile, one pair was offered a cut leaf of garlic mustard in a small plastic cup (6.5 cm diameter, 7cm high) for 2-3 days. Only females that laid eggs were used in the tests. In addition, all weevils released were marked with a spot of nail varnish on the elytra to be able to distinguish between them from weevils emerging from plants. On 29 October and 1 November 2002, plants were thoroughly searched for weevils and any signs of feeding or oviposition were recorded. Then plants were re-covered with gauze bags and placed in the Centre's garden for overwintering. All replicates established with *Peltaria alliacea* and two controls were kept in a greenhouse during the winter since this test plant species is not frost hardy. The remaining test plants and controls were kept in the Center's garden. Plants will be searched regularly for emerging weevils from late spring 2003 onwards.

Multiple-choice oviposition and larval development test
On 31 October 2001 a multiple-choice cage test (2 x 2 x 2m) was established with *Barbarea vulgaris, Brassica oleracea sebellica, Brassica oleracea sabauda* "Eiskönig", *Rorippa amphibia,* and garlic mustard. All plants were overwintered in the Center's garden. Between 17 May and 13 July 2002, plants were regularly searched for emerging weevils, and their number and sex recorded. On 13 July, plants were dissected and signs of larval mining recorded. Plants of *R. amphibia* were not dissected, because they had produced viable seeds and were needed for *Ceutorhynchus constrictus* development tests.

Results

Sequential no-choice oviposition tests
A Total of 47 plant species was tested plus 8 varieties of *Brassica oleraceae.* Surprisingly, *C. scrobicollis* laid eggs on 23 of these species and on 6 of the 8 varieties (Table 13). However, eggs recorded on *Phlox divarica*, *Viola sororia* and *Hydrophyllum virginianum*, all native North American plant species, were laid on the outside of the plant material. We interpret this unusual oviposition behavior as a lab artifact. Further indication for a lab artifact is that no adult feeding was observed on these plant species. In fact it is known from many other similar studies that females with maturing eggs, simply need to lay eggs somewhere, creating such unusual conditions. All other test plants accepted for oviposition were within the family Brassicaceae. Eggs were recorded on five species endemic to North America, *Cakile edentula, Draba reptans, Dentaria heterophylla, D. laciniata,* and *Lepidium virginicum*. In general, females laid fewer eggs into test plants than the control, except for *C. edentula, Thlaspi arvense* and *Peltaria alliacea*, into which females laid the same number or more eggs than into *A. petiolata*. All three test plant species had been kept in a greenhouse in order to protect them from attack by oligophagous insects occurring naturally in the Centre's garden, while garlic mustard had been kept in an outdoor field cage. Therefore, their tissue was softer than that of garlic mustard, which might partly explain why they were so readily accepted for oviposition by *C. scrobicollis* (also see results for *C. alliariae* and *C. roberti*). However, no eggs were laid into *Sysimbrium irio*, the third species included because of its

relatedness to garlic mustard. *Ceutorhynchus scrobicollis* did not attack another closely related species, *Tropaeolum majus,* a species tested because it contains glucosinolates, a known feeding and oviposition stimulant for other weevils of Cruciferous host plants.

Single-choice oviposition tests

Females of *C. scrobicollis* accepted 14 species for oviposition including three species indigenous to North America, *Draba reptans, Cakile edentula* and *Lepidium virginicum* and also 3 varieties of *Brassica oleraceae* (Table 14). However, only two and one egg were laid on *D. reptans* and *L. virginicum,* respectively. Similar to results of no-choice oviposition tests (see above), an equal or even higher number of eggs were laid into *Thlaspi arvense* and *Peltaria alliacea* compared to the control.

No-choice oviposition and larval development test

All four garlic mustard rosettes established as controls in 2001 were attacked and on average, 16 weevils emerged from each replicate (Table 15). No weevils emerged from any of the test plant species exposed, and no signs of larval mining attributable to *C. scrobicollis* were detected, not even on the *B. oleracea sabauda* variety offered, although one adult had emerged from another *sabauda* variety in 2001. In 2002 tests, of 105 weevils released, 94 (89.5%) were recovered; 1-2 females could not be detected on four plants. Feeding was observed on all test plant species and the control. Results on weevil emergence will be recorded in summer 2003.

Multiple-choice oviposition and larval development test

All five *A. petiolata* plants exposed were attacked and on average 19 *C. scrobicollis* adults emerged from each plant (Table XX). No weevils emerged from any of the test plant species exposed, and no signs of larval mining attributable to *C. scrobicollis* were detected.

A complicating factor in our evaluations was the fact that test plants kept in the Centre's garden prior to tests, were often attacked by a polyphagous weevil (probably *Ceutorhynchus pictitarsis*, identification pending). This weevil was successfully reared from 4 plant species (*Armoracia rusticana*, *Barbarea vulgaris*, *Brassica nigra* and *Brassica oleracea gemmifera*). Mining found in several plants could therefore not always be clearly attributed to *C. scrobicollis*. In addition, individuals of *C. scrobicollis* were recovered from several replicates, although no mining was found. These individuals were most likely weevils which were overlooked when adults were retrieved from test plants. Due to these ambiguous results, tests were repeated for *Arabis laevigata, Armoracia rusticana*, *B. oleracea gemmifera*, *Barbaraea vulgaris*, *Brassica napus var napus*, *Brassica oleracea sabauda*, and *Rorippa amphibia*. To avoid the above-mentioned problems, test plants were kept as long as possible in the greenhouse or cages prior to tests (albeit this softens tissue and may explain some positive test results), and all *C. scrobicollis* released on plants for oviposition were marked with a white dot on the elytra enabling us to distinguish these individuals from newly emerged ones.

While we are still missing a number of tests, the evidence accumulated so far indicates that the root-feeder *C. scrobicollis* is a primary candidate as a biological control agent.

While quite a number of test-plant species are used for oviposition by females in our testing sequence, as soon as tests become more realistic in design, oviposition rates drop. Moreover, larval development appears restricted to the original host plant, garlic mustard.

Phyllotreta ochripes

The flea beetle *P. ochripes* is (in contrast to the *Ceutorhynchus* species) described as oligophagous (attacking more than a single plant species) in the literature. In addition to attacking garlic mustard, the species has been recorded from *Rorippa* spp. We used two different tests, (a) no-choice larval transfer tests and (b) multiple-choice oviposition and larval development test in field cages to test the host-specificity of *P. ochripes*.

Methods

No choice larval transfer test

Pairs of *P. ochripes* were provided with cut leaves of garlic mustard inserted into moist florist foam. Females used the foam as oviposition substrate and eggs were collected from the foam by removing the upper 0.5 cm layer at 4-5 day intervals. Eggs were incubated at room temperature in Petri-dishes and checked twice daily. Newly hatched first instar larvae were transferred with a fine paint-brush onto potted plants of garlic mustard (=control) and onto test plant species (N= 30 larvae/plant). Plants were kept in the laboratory for two days, and then placed into a gauze-covered cage in a common garden. After 6 weeks, plants were individually covered with gauze bags and placed into an open unheated greenhouse. Every 7-10 days, plants and bags were carefully searched for *P. ochripes* adults.

Multiple-choice oviposition and larval development test

A multiple-choice cage test was established with three *Rorippa* species (*R. amphibia, R. palustris,* and *R. sylvestris*) and garlic mustard on 6 May 2000. Four individually potted plants of each of the four plant species were arranged in a Latin Square design in a gauze-covered field cage (2 x 2 x 1.6 m), and 32 pairs of *P. ochripes* were released. Plants were removed from the cage on 20 June 2000, individually covered with gauze bags, and placed into an open greenhouse for adult emergence. Pots and bags were checked at weekly intervals. We repeated the field cage experiment during the 2001 field season using plant species that supported development under no-choice conditions, particularly plants of economic importance. On 18 May 2001, a multiple-choice cage test was established using *Barbarea vulgaris*, *Brassica napus*, *Brassica nigra*, *Nasturtium vulgaris*, *Raphanus sativus*, *Sinapis alba* and garlic mustard. Five individually potted plants of each of the four species (i.e. 35 plants in total) were arranged in 5 rows in a gauze-covered field cage (2 x 2 x 1.6 m). Within each row, plants were arranged randomly. We released 32 pairs of *P. ochripes* and on 11 June, plants were removed from the cage, individually covered with gauze bags, and placed into an open unheated greenhouse. From 6 July until 10 September, plants were checked every 4-11 days for adult emergence.

Table 16. Results of larval transfer tests with *Phyllotreta ochripes*.

Family Tribe Plant species	# plants established	# plants from which adults emerged	# larvae transferred	# adults emerged	% survival (mean ± SE)
Brassicaceae					
Arabideae					
Alliaria petiolata	38	34	1140	281	24.6
Armoracia lapathifolia (= *A. rusticana*	2	0	60	0	0
Barbarea vulgaris	5	5	180	30	16.7
Nasturtium vulgaris	5	5	150	46	30.7 ± 10.5
Rorippa amphibia	3	3	90	28	31.1 ± 5.9
Rorippa palustris	2	2	60	14	23.3
Rorippa sylvestris	5	4	150	21	15.3 ± 5.3
Alysseae					
Hesperis matronalis	5	0	150	0	0
Brassiceae					
Brassica napus	4	3	120	9	7.5
Brassica nigra	5	3	150	6	4.0±0.9
Brassica oleracea gemmifera	5	0	150	0	0
Brassica oleracea italica	5	0	150	0	0
Raphanus sativus	4	2	120	4	3.3
Sinapis alba	5	1	150	8	5.3 ± 5.3
Resedaceae					
Reseda lutea	3	1	90	1	1.1 ± 1.1
Poaceae					
Hystrix patula (= *Elymus hysterix*)	5	0	150	0	0
Zea mays	5	0	150	0	0
Triticum aestivum	5	0	150	0	0
Fabaceae					
Glycine max	5	1	150	1	0.7 ± 0.7
Liliaceae					
Smilacina racemosa	3	1	90	1	1.1 ± 1.1
Araceae					
Arisaema triphyllum	5	0	150	0	0

Table 4 (continued)

Family Tribe Plant species	# plants established	# plants from which adults emerged	# larvae transferred	# adults emerged	% survival (mean ± SE)
Aristolochiaceae					
Asarum canadensis	5	0	150	0	0
Apiaceae					
Osmorhiza claytonii	5	1	150	1	0.7 ± 0.7
Rubiaceae					
Galium aparine	5	0	150	0	0
Papaveraceae					
Sanguinaria canadensis	5	0	150	0	0
Geraniaceae					
Geranium maculatum	5	0	150	0	0
Berberidaceae					
Podophyllum peltatum	5	0	150	0	0
Hydrophyllaceae					
Hydrophyllum virginianum	5	1	150	2	1.3 ± 1.3
Vitaceae					
Parthenocissus quinquefolia	5	1	150	1	0.7 ± 0.7
Asteraceae					
Solidago flexicaulis	5	0	150	0	0
Polemoniaceae					
Phlox divaricata	5	0	150	0	0

Results

No choice larval transfer test

Between 3 May and 17 June 2000, 5,250 larvae were transferred onto 178 plants; 40 of garlic mustard, and 138 of 30 different test plant species (2-5 plants/species). On garlic mustard about 24% of transferred larvae developed into adults within 7-8 weeks. Apart from garlic mustard, 15 other plant species supported the development of *P. ochripes* (Table 16). However, from six of these (*Reseda lutea, Glycine max, Smilacina racemosa, Osmorhiza claytonii, Hydrophyllum virginianum*, and *Parthenocissus quinquefolia*) only 1-2 adults emerged and no signs of larval mining were found upon dissection. All plant species supporting sustained levels of larval development of *P. ochripes* (*Nasturtium vulgaris*, *Rorippa amphibia*), are members of the same tribe as *A. petiolata*, Arabideae. Larval survival on the commercially grown *Brassica napus*, *B. nigra, Raphanus sativus* and *Sinapis alba* was limited; development was not supported on the two *Brassica oleracea* cultivars (Table 16).

In test conducted during 2000, adults emerged from all three *Rorippa* species exposed, although garlic mustard was clearly preferred (Table 17). Extremely high numbers of adults emerged from garlic mustard, indicating some severe oviposition pressure. In fact, all four garlic mustard plants died soon after they were put under gauze. This highlights the potential of *P. ochripes* as a biological control agent for garlic mustard. During test conducted in 2001, adults emerged from three test plant species exposed. Two of these, *Brassica nigra* and *Sinapis alba* are commercially grown crucifers. From *Nasturtium vulgaris*, more adults emerged than from garlic mustard (Table 17).

Table 17. Results of multiple-choice field cage test with *Phyllotreta ochripes* in 2000 and 2001.

Plant species	No. plants exposed	No. plants from which adults emerged	Total no. adults emerged	Mean no. adults emerged/plant (± SE)
2000				
Alliaria petiolata	4	4	493	123.3 ± 26.1
Rorippa amphibia	4	3	16	5.3 ± 2.9
Rorippa palustris	4	4	23	5.8 ± 2.1
Rorippa sylvestris	4	4	36	9.0 ± 2.2
2001				
Alliaria petiolata	5	5	47	9.4 ± 4.8
Barbarea vulgaris	5	0	0	0
Brassica napus	5	0	0	0
Brassica nigra	5	3	11	3.7 ± 0.9
Nasturtium vulgaris	5	5	52	10.4 ± 2.5
Raphanus sativus	5	0	0	0
Sinapis alba	5	1	3	0.6

These host specificity results for *P. ochripes* are a clear indication that the species is not sufficiently host specific to be considered for introduction to North America. Although the species is clearly damaging to garlic mustard, we have stopped considering this flea beetle a potential biological control agent.

Recommendations for selection of agents and future work

The seed feeding weevil, *Ceutorhynchus theonae*, was too rare, and rearing under quarantine conditions in Switzerland too difficult, to pursue investigations of this insect. A second species, the flea beetle *Phyllotreta ochripes*, showed promise based on multiple generations and significant impact on plant performance. However, the species attacked a number of other plant species, even in multiple-choice feeding tests, and therefore is not considered sufficiently specific to be considered further as a biological control agent.

We were unable to complete the entire sequence of all 50 plant species proposed for testing during the 3 years of this project, although the vast majority of plants was tested for all 4 weevil species currently considered as potential biological control agents. While we were unable to complete the entire testing sequence for all control agents, we are well on our way to complete all host specificity work and to file a petition with TAG within the next 12-15 months (at least for *C. scrobicollis*). Detailed investigations of the seed-feeding weevil *Ceutorhynchus constrictus*, two stem mining weevils, *C. alliariae* and *C. roberti*, and a stem and root-crown feeding weevil *C. scrobicollis* produced abundant information on life-history, ecology, and host specificty. The seed feeder was widely distributed in Europe but attack rates remained fairly low throughout the investigative period. This may be, in part, explained by significant mortality through attack by parasitic natural enemies. Experiments showed that the two stem-mining weevils are reproductively isolated species that can co-exist in the same ecological niche. However, their impact on garlic mustard performance in experiments was not particularly dramatic resulting in little reductions in biomass or seed output. Field observations suggest that a larger impact can be expected of these species once released in North America. Problems in experimental design (by limiting the ability of females to move freely among plants) may have created intense competition for oviposition sites. The most promising and significant impact was observed by attack of *C. scrobicollis*, a species active in fall, winter and spring. Attack by this root-mining weevil reduced plant survival, plant biomass and seed output, key variables in plant population demography of garlic mustard.

At present we consider all four species investigated in detail as viable candidates for release in North America. n particular the root feeder *C. scrobicollis* appears to be a species with considerable potential to reduce performance and abundance of *A. petiolata* after release in North America. As an additional safety precaution, we will need to complete additional test in Europe and under quarantine in North America for a number of North American Brassicaceae. We have been able to get funding support from the US Forest Service for this work and additional tests are planned for a newly opened quarantine facility in Minneapolis, MN beginning in 2003. A team will visit CABI in March 2003 to learn methods of testing and rearing and the initial focus will be *C. scrobicollis*.

Natural enemies of garlic mustard in North America

Introduction

We surveyed for potential natural enemies of garlic mustard in the introduced range to determine whether native (or introduced) natural enemies are already present in North America. Preliminary observations in the Northeast and Midwest suggested that a number of species could attack garlic mustard in the introduced area. Among these were generalists like *Pieris rapae*, spittlebugs, and stem-mining weevils and a stem-mining fly. Biotic resistance of native species attacking an introduced plant may contribute to reduced invasiveness. Introduction of non-indigenous insects is a common phenomenon and it is at least conceivable that some of the European natural enemies of garlic mustard may have been introduced accidentally to North America. In a related effort, investigating potential natural enemies of *Phragmites australis*, surveys have documented the establishment and proliferation of over a dozed European herbivores in North America over the last decades (Tewksbury et al. 2002). Much effort on screening for specificity and introduction of potential control agents could be wasted if some of the European natural enemies of garlic mustard were already established in North America.

Methods and Materials

To gain a better sense of the distribution and severity of the attack, we established a standardized collection protocol, and requested samples from collaborators across the known distribution of the species in North America. The protocol asked for collection of $0.25m^2$ samples (0.5 by 0.5m; removing all garlic mustard plants and their roots) established along a transect across a garlic mustard population. Based on preliminary results on peak activity of herbivores, we requested the sampling to occur when the majority of the plants stopped flowering. The distances between samples varied by site depending on the size of the population. We requested that collaborators submit at least 5 samples from each site with information on spatial arrangement of the samples. By requesting transect samples we tried to eliminate the tendency to collect in dense patches or along edges of populations. We were particularly interested whether any features such as number of stems, or size of plants would influence herbivore attack rates. Plant samples were wrapped in plastic bags to reduce moisture loss and were mailed using overnight carriers to Cornell University. Samples that could not immediately be processed were stored at 5°C in a walk-in refrigerator.

We developed a standardized dissecting protocol to record plant parameters (number of plants/$0.25m^2$, number of stems per plant, stem height, stem diameter at the base of the stem, and reproductive status). Each plant and each stem, root-crown and root was then carefully examined and dissected to assess attack by insects or fungi. We recorded presence or absence of attack when we were unable to attribute the attack to a certain organism (siliques, root, root-crown, leaf feeding). We counted the number of leaf-mines on each stem and the number of fly and weevil larvae attacking each stem. In addition we scored the severity of stem attack (0=no attack; 1 light attack, approx. 1/3 of the stem mined; 2=medium, approx. 2/3 of the stem mined; and 3=severe, nearly all of the stem mined). We removed all larval instars and all leaf-mines with larvae from each plant and kept them separate by species in Petri dishes or small containers. Externally feeding herbivores were provided with fresh leaf pieces every few days until pupation. Weevil and fly larvae were provided with fresh stem pieces and larvae were inoculated into stems to allow for larval development. In regular intervals (usually every 2-3 days) all containers were checked for emerging adults and herbivores and their parasitoids were stored in alcohol for later taxonomic analysis. We have developed a filemaker database to store, track and analyze the data. Remaining soil was washed off roots and all plant material from each quadrat was placed in paper bags and dried to constant weight at 80°C in a drying oven and dry biomass was recorded to 0.01g precision.

Results

Please note that the results presented in the following are the first summaries of the large amount of data represented in our database. We dissected 1,423 garlic mustard stems collected at field sites in NY, PA, and Il in 2000 and 5,022 stems in 2001. Dissection protocols differed slightly between the two years and we will merge the two databases in the future. A more extensive analysis is forthcoming and additional stem dissections need to be entered.

We received and dissected over 5,000 stems in samples collected in May and June 2001 from 15 different states representing over 40 garlic mustard populations (Table 18), including samples from military installations (West Point, Ft. Custer Training Center, Arnold Air Force Bay, Ft. McCoy, and Fermi Lab). The large number of samples allowed us to explore relationships of plant growth and insect attack across the country. The number of stems/0.25m^2 at each site was very variable and ranged form 1 to >80 with no clear pattern (Fig. 29). There was no gradient from South to North or East to West; within each region variations were as large as across the entire range of sites examined (Fig. 29). The same pattern was found for the amount of biomass/0.25m^2, and please note that the number of stems is not necessarily a good predictor for the amount of biomass produced (Fig. 30). Mean stem height/0.25m^2 was more consistent than the number of stems or biomass and at the majority of the sites mean stem height fell between 50 and 100cm (Fig. 31). As for the other plant parameters, there was no geographic trend across North America. Stem height and stem diameter were tightly correlated (Fig. 32) throughout the size range of the plant.

In our analysis of insect attack we calculated all attack rates as the mean of the mean attack rate per sampling quadrat (0.25m^2). We were surprised to find that some of the initial observations from the 2000 field season could not be confirmed in 2001. For example, we found an abundance of weevil larvae at several sites in the East in 2000, yet in 2001 attack rates were generally very low, thus limiting our ability to make inferences about habitat features and attack rates during 2001. The only species with abundant attack was a leaf mining fly (taxonomic identification still pending). The species was regularly, although in low abundance, recorded at sites in the Northeast in 2000, yet in 2001 the

Table 18.. State, location, date of collection, and the number of sampling quadrats collected for analysis of garlic mustard herbivores in North America during the 2001 field season.

State	Location	Date	# of quadrats
DC	George Washington Memorial Parkway	22 June 2001	2
IL	Aldeen Park	22 June 2001	10
IL	Chicago Botanic Garden 1	24 may 2001	6
IL	Chicago Botanic Garden 2	7 June 2001	5
IL	Fermilab	27 May 2001	5
IL	Glenview Woods	4 June 2001	2
IL	Hall Woods	22 June 2001	10
IL	Hart Woods	24 May 2001	5
IL	Illinois State Beach	21 May 2001	5
IL	Max McGraw	6 June 2001	10
KY	Boone County Cliffs Preserve	9 May 2001	10
KY	Tom Dorman NP	14 May 2001	51
KY	Manchester 2 Island	17 May 2001	8
MA	Lincoln Park	28 May 2001	3
MA	Silvio NWR (Poet's Seat Tower)	23 May 2001	6
MI	Utes Road (Ft Custer Training Center)	12 June 2001	5
MN	Afton Trout Brook	1 June 2001	5
MN	Blaine White Farm	8 June 2001	3
MN	Fort Snelling Park	19 June 2001	4
MN	Snelling Lake Trail	31 May 2001	10
MN	Wood-Rill	19 June 2001	7
NJ	West Trenton	31 May 2001	5
NJ	Walkill	5 June 2001	5
NY	Boquet Liquor Store	27 June 2001	5
NY	Leon	14 June 2001	5
NY	Mashomack	5 June 2001	5
NY	Muttontown	31 May 2001	5
NY	Sag Harbor	22 June 2001	5
NY	Stewart Airport	16 June 2001	5
NY	Suny Canton	10 June 2001	5
NY	Uplands Farm	15 May 2001	5
NY	West Point Military Academy	17 June 2001	13
OH	E Badger Farm	21 May 2001	5
OH	Secor Metro park	20 May 2001	6
ONT	Cochrane Rd	13 June 2001	6
ONT	High Park 3B	30 May 2001	5
ONT	UWO campus	25 June 2001	9
OR	Tryon Park	29 May 2001	6
PA	Delaware Water Gap	17 June 2001	10
TN	Arnold Airforce	10 May 2001	5
VA	Blue Ridge Parkway	30 May 2001	1
WA	Carkeek Park	17 May 2001	4
WI	Fort McCoy	30 May 2001	6
WI	Kern Park	27 June 2001	5
WI	Kettle Moraine	19 June 2001	5
WI	Lake Kegonsa 1	12 May 2001	5
WI	Lake Kegonsa 2	22 June 2001	5
WI	Lulu Lake	29 May 2001	5
WV	Grape Island	21 May 2001	8

species was entirely absent from sites in the Northeast but particularly abundant at several sites in Illinois, Wisconsin and Minnesota (Fig. 32). At several sites we found densities of several hundred mines/0.25m^2 but this did not seem to affect the performance of garlic mustard or the ability to produce fertile seed.

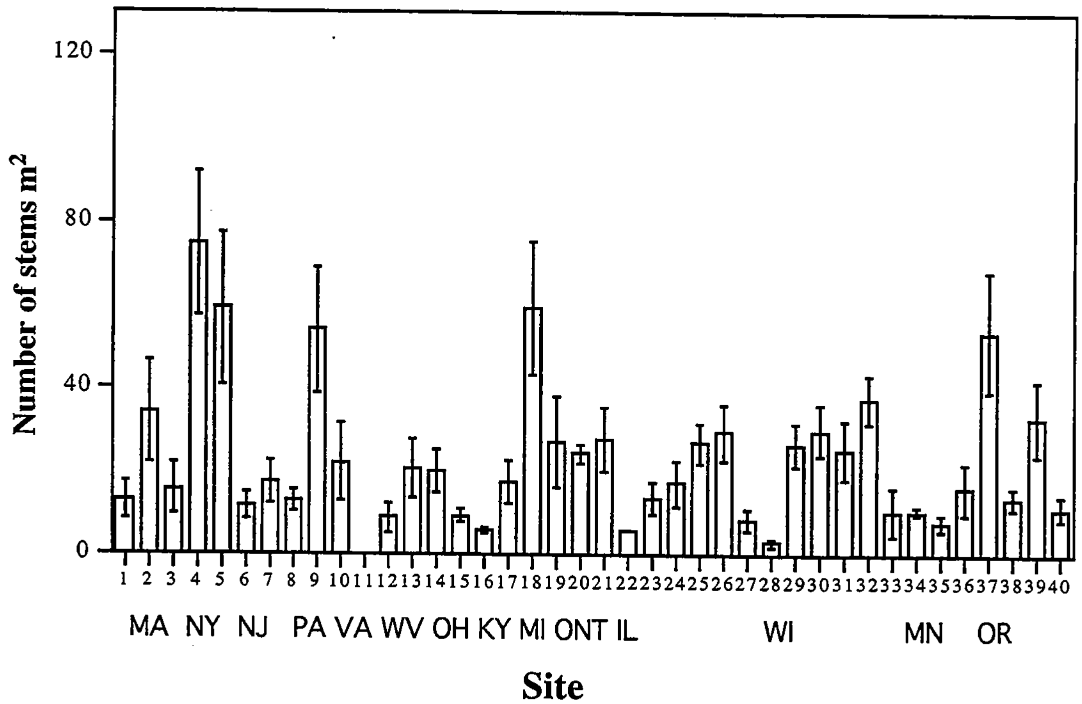

Fig. 29. Number of garlic mustard stems/0.25m^2 at each of 40 collection sites in North America. State codes below the x-axis represent approximate locations of the samples ordered from East to West. Data are means (±SE) of 3-10 samples at each location.

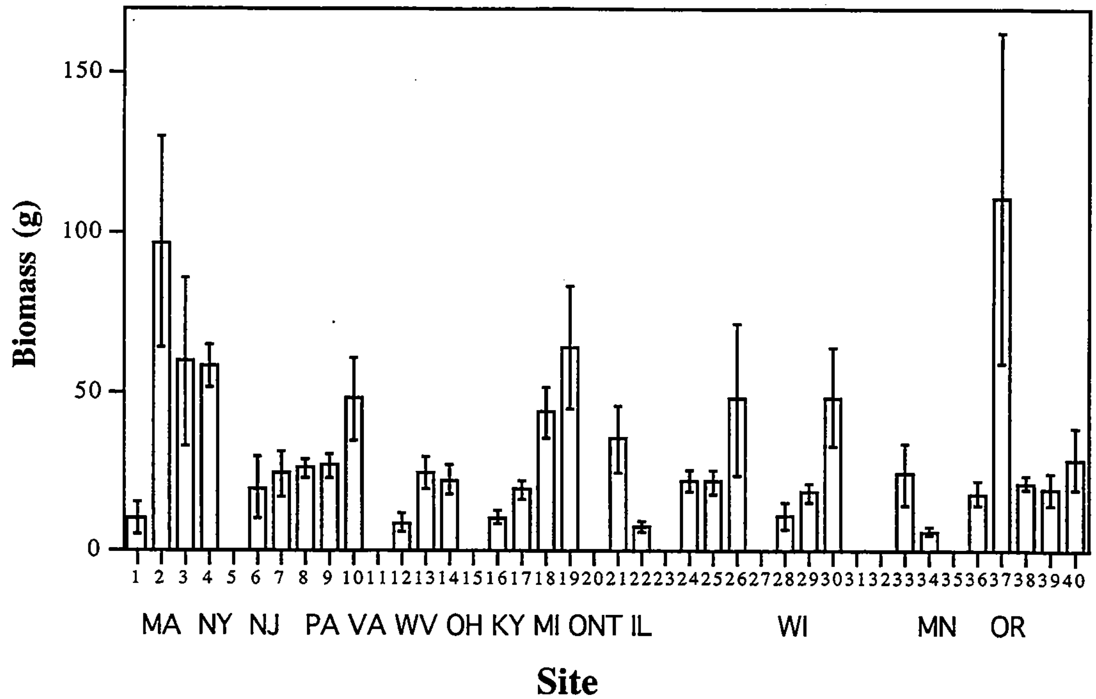

Fig. 30. Garlic mustard biomass/0.25m^2 at collection sites in North America (biomass was not recorded at all sites). State codes below the x-axis represent approximate locations of the samples ordered from East to West. Data are means (±SE) of 3-10 samples at each location.

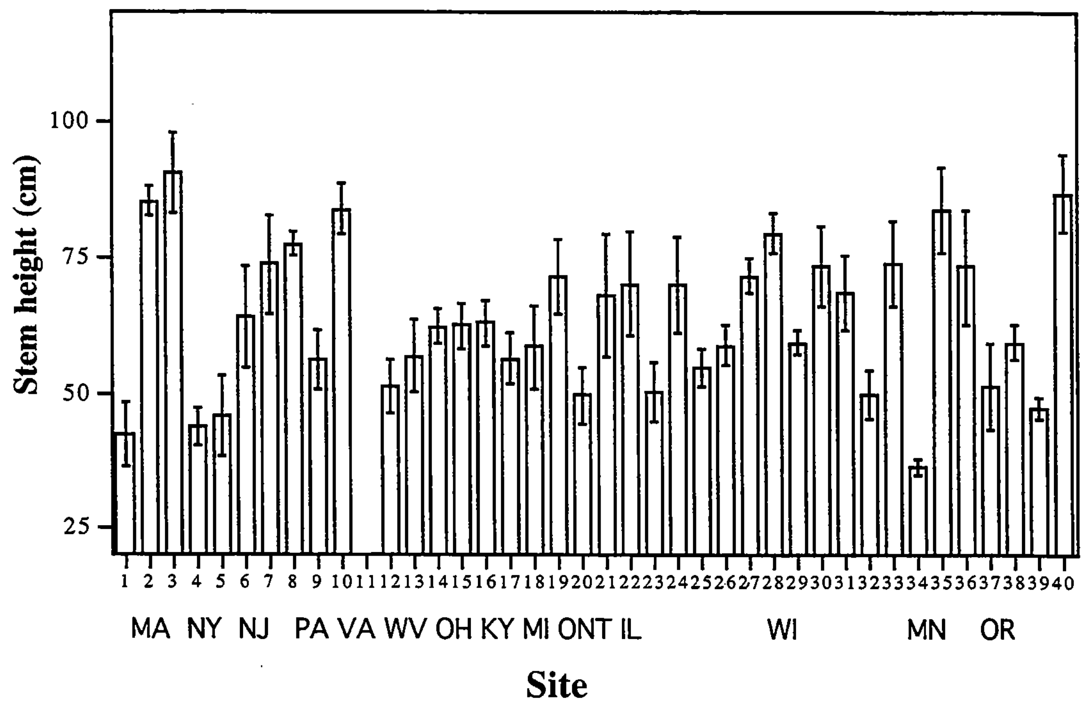

Fig. 31. Garlic mustard stem height/0.25m^2 at each of 40 collection sites in North America. State codes below the x-axis represent approximate locations of the samples ordered from East to West. Data are means (±SE) of 3-10 samples at each location.

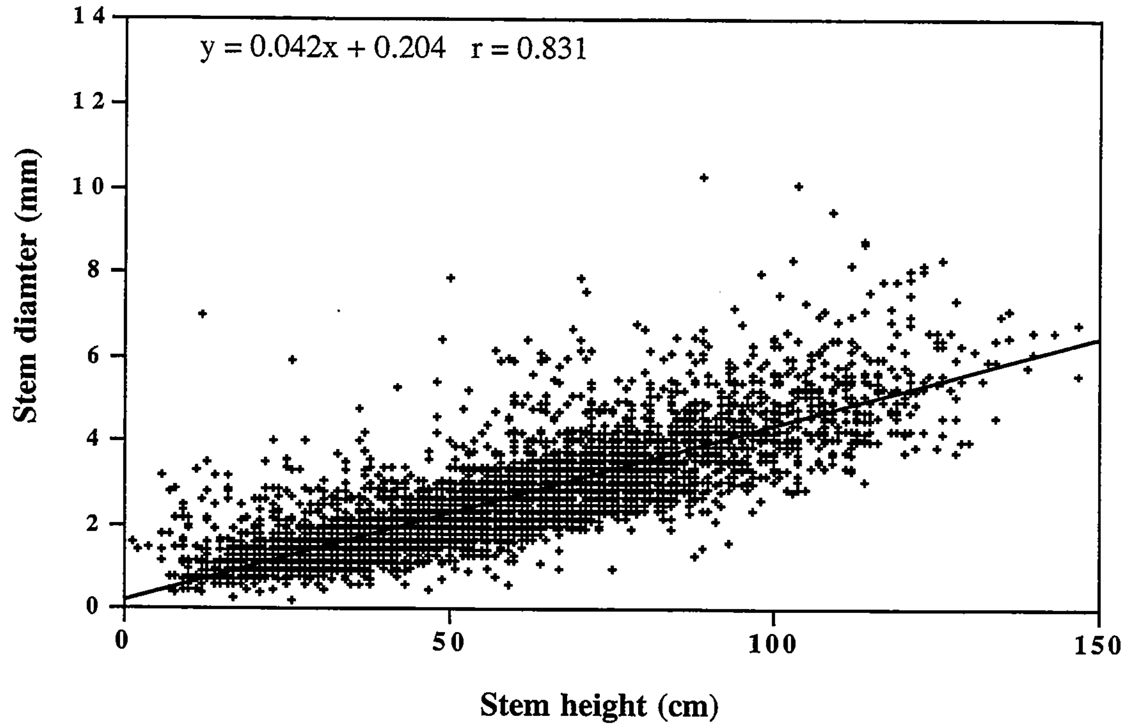

Fig. 32. Relationship of stem height (cm) and stem diameter (mm) for garlic mustard plants collected in May and June 2001 across North America (N=5022 stems).

The abundance of this species allowed us to explore a few relationships of attack rates with garlic mustard abundance. We only incorporated sites where the fly was present in the following analyses. Flies preferred to attack taller plants and the he number of leaf mines/stem increased exponentially (Fig. 34); plants under 30cm were only rarely attacked by the leaf miner. Highest attack rates on tall plants reached 30 or 40 mines/stem (Fig. 34). However, attack rates/stem (Fig. 35) and attack rates/0.25m^2 (Fig. 36) declined with increasing stem density of garlic mustard. In many of the rearings we commonly encountered a hymenopteran parasitoid attacking fly larvae and pupae. High parasitoid attack rates on leaf miners are not unusual and it appears that a single species of solitary parasitoid is attacking this fly across the entire North American range. The species status of this parasitoid is still under investigation. The rarity at field sites in the Northeast (despite extensive searches in 2001, we could not find leaf mines at locations where the leaf miner was previously common) may also be a function of parasitoid activity. Boom and bust cycles of leaf miners with parasitoids causing 100% mortality are described for a number of biocontrol systems and fairly common (Hawkins and Cornell 1999). We expect the leaf miner to have several generations/year. Many mines on the lower parts of tall plants were already empty and flies continued to emerge from mines collected in May and June. We were able to induce a new generation of flies by releasing emerging adults into a field cage with potted garlic mustard plants in a common garden. The species may not be a garlic mustard specialist since we did not find any indication for initiation of dormancy even when plants were senescing.

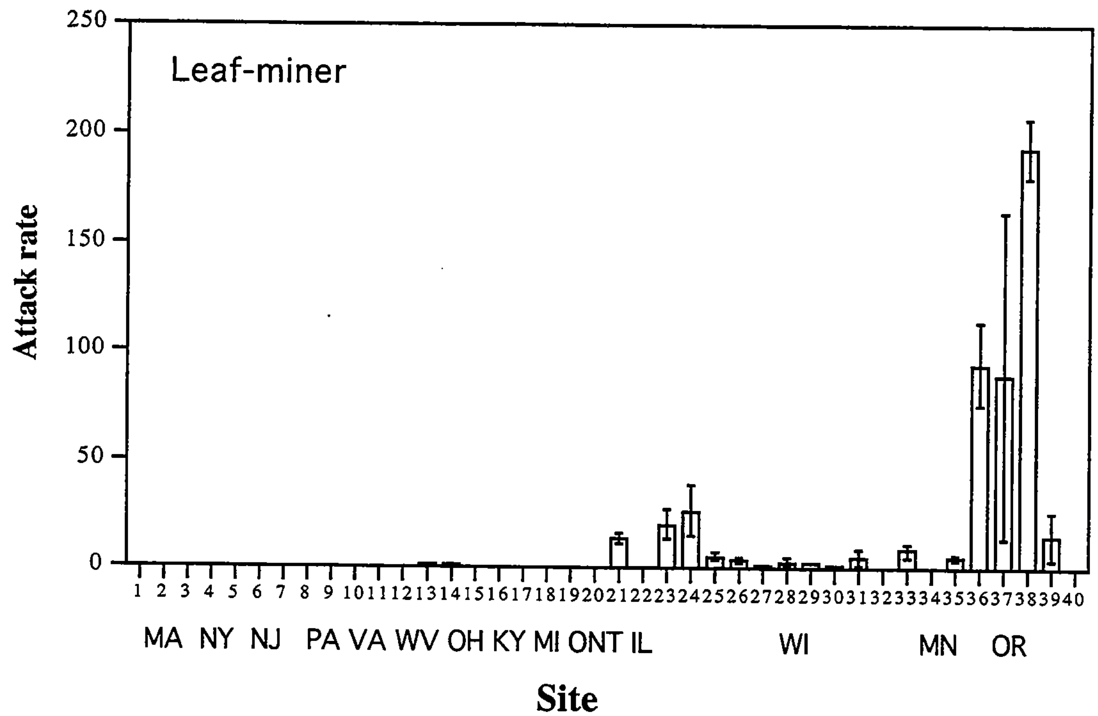

Fig. 33. Attack rates (number of leaf-mines/0.25m^2) on garlic mustard at 40 collection sites in North America. State codes below the x-axis represent approximate locations of the samples ordered from East to West. Data are means (±SE) of 3-10 samples at each location.

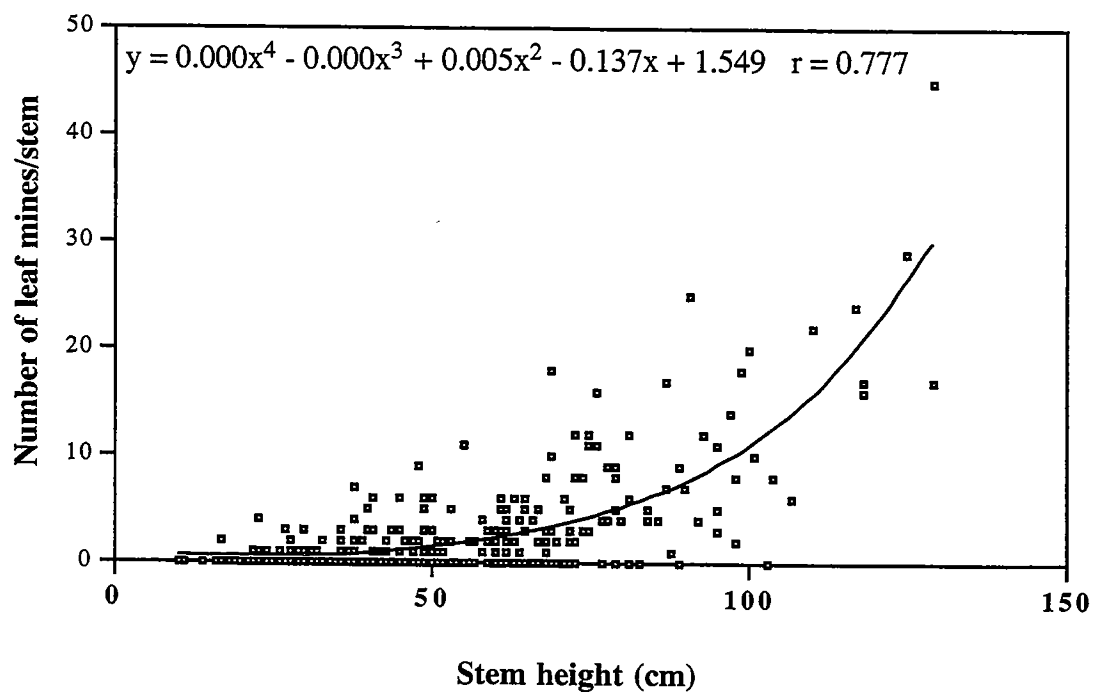

Fig. 34. The number of leaf-mines/stem of garlic mustard as a function of stem height.

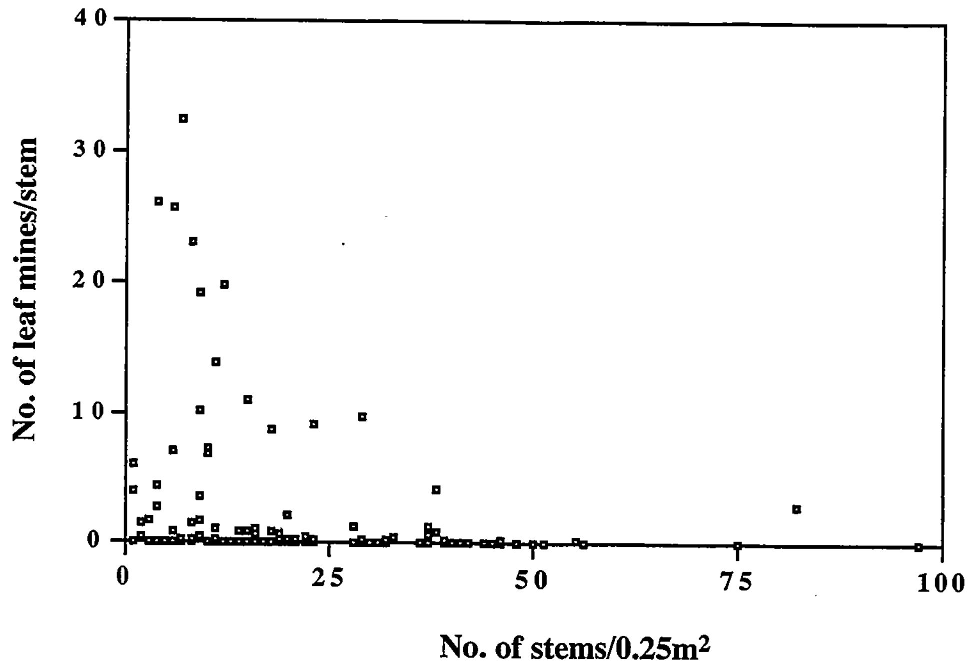

Fig. 35. The number of leaf-mines/stem as a function of the number of garlic mustard stems/0.25m^2.

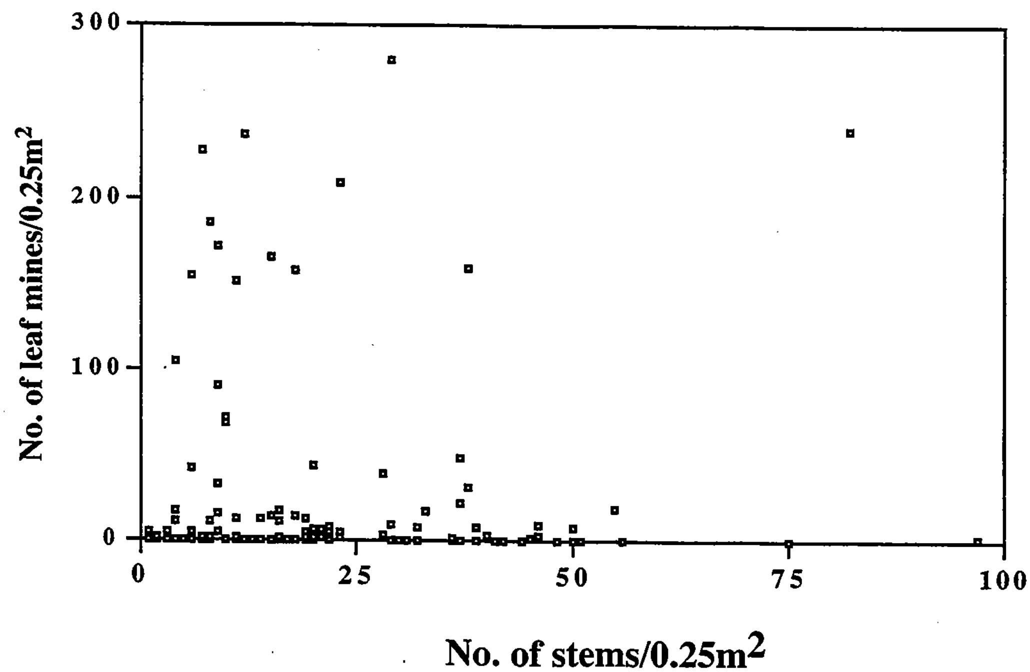

Fig. 36. The number of leaf-mines/0.25m^2 as a function of the number of garlic mustard stems/0.25m^2.

A small fly larva was a regular but uncommon occurrence in dissected garlic mustard stems during the 2000 and 2001 field seasons. We often found the larvae associated with stem mining weevil larvae (see below) and occasionally 2-4 larvae were found in the lower third of attacked stems. The feeding damage did not offer any immediate clues of whether the larva is solely herbivorous or omnivorous (potentially feeding on weevil feces and decaying plant material in the garlic mustard stems). It appears that the species (at least when attacking garlic mustard) is univoltine and overwinters as pupa in the stem. While the species was never abundant, it was a regular occurrence at field sites in the Northeast (Hudson River, Finger Lakes Region) during 2000. At West Point, attack rates reached 2.9±1.7% of stems in 2000 but only plants in the shade were attacked. In the 2001 stem collection from across North America, the species was only recorded at 4 field sites in the Midwest and in extremely low abundance (Fig. 37). Garlic mustard stems were collected in the fall 2001 at Waterman Conservation Tract near Binghamton, NY, where the species was present in 2000. Preliminary stem dissections revealed that fly pupae were present in the fall and approx. 100 stems were overwintered in gauze bags in a sheltered outdoor location. In the spring, stems were placed into plastic bags and kept under room temperature to capture emerging adults for species identification in the spring. Unfortunately, only a single fly emerged, the remaining 30 adults were parasitoids. Species identification for both the fly and its parasitoid is pending. The impact of the fly on garlic mustard performance is negligible.

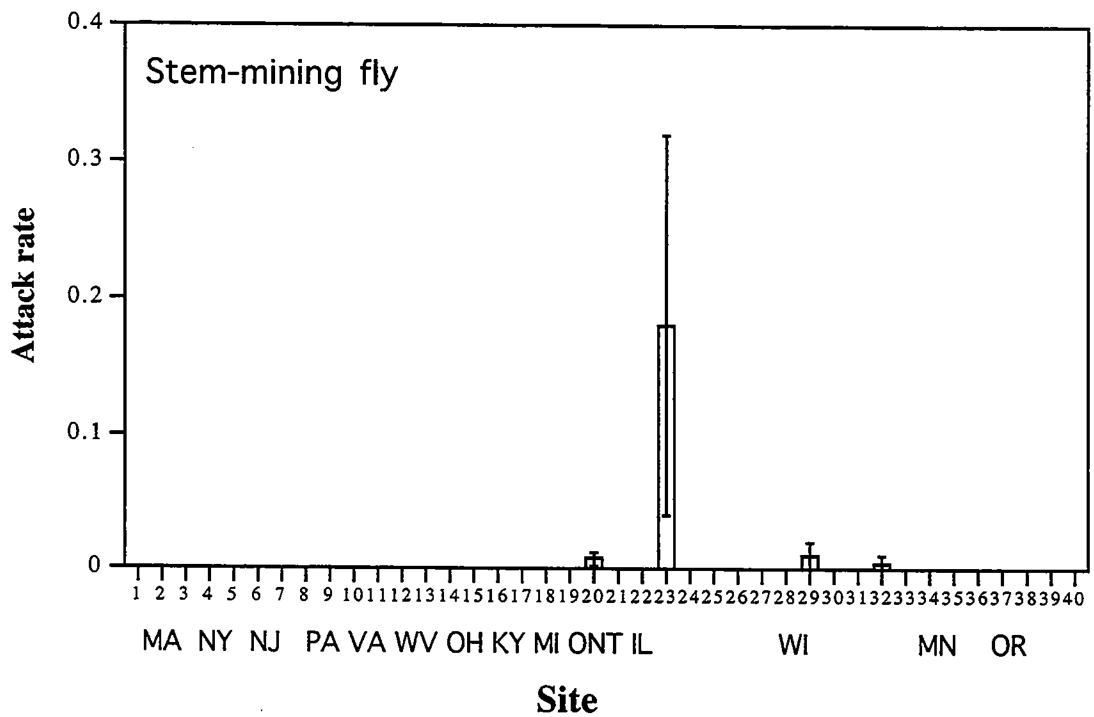

Fig. 37. Attack rates (proportion of stems attacked) of a stem mining fly at each of 40 collection sites in North America. State codes below the x-axis represent approximate locations of the samples ordered from East to West. Data are means (±SE) of 3-10 samples at each location.

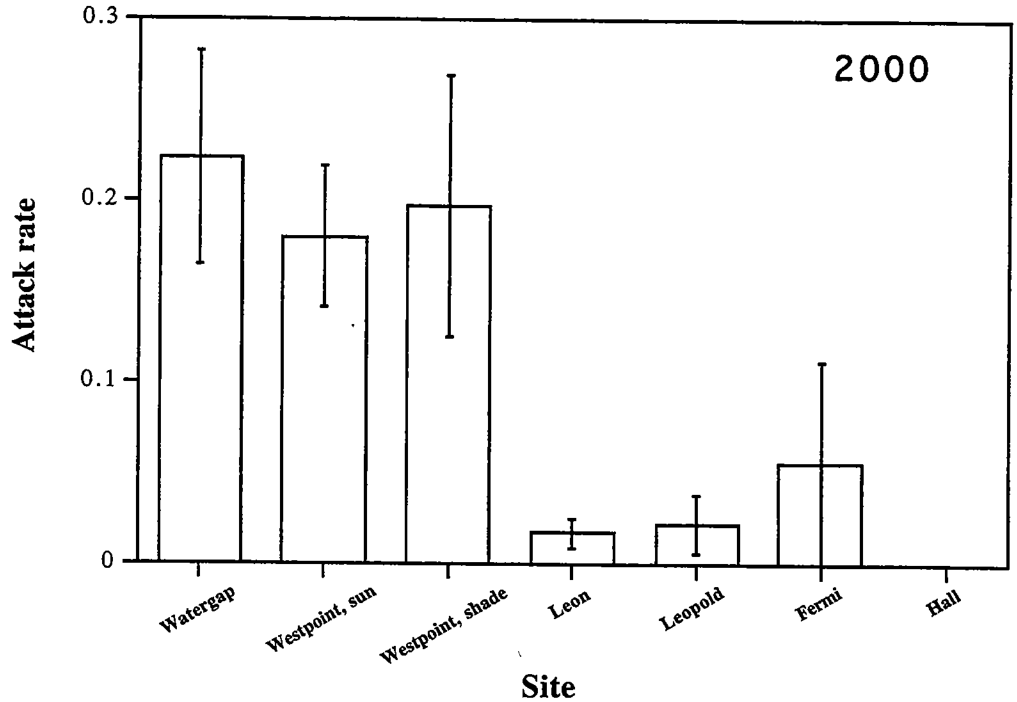

Fig. 38. Attack rates (proportion of stems attacked) of a stem-mining weevil at 7 collection sites in the Northeast and Midwest of North America. Data are means (±SE) of 5-13 samples (0.25m^2) at each location.

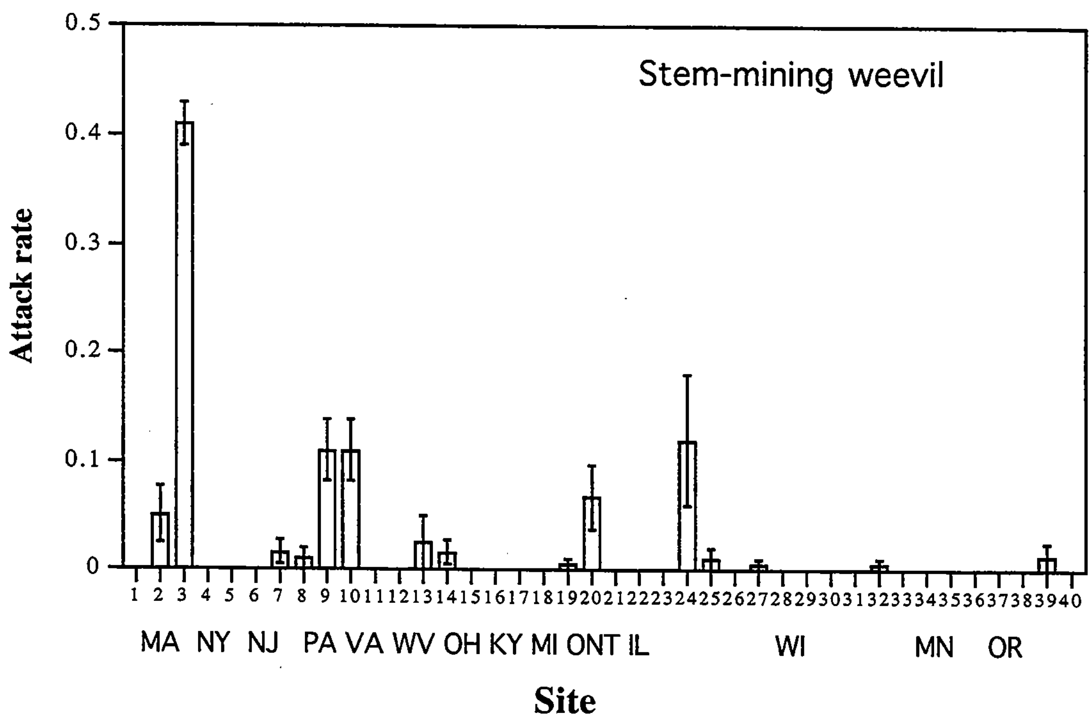

Fig. 39. Attack rates (proportion of stems attacked) of a stem-mining weevil at each of 40 collection sites in North America. State codes below the x-axis represent approximate locations of the samples ordered from East to West. Data are means (±SE) of 3-10 samples at each location.

The most common herbivore on garlic mustard in our 2000 stem dissection was a stem-mining weevil, *Ceutorhynchus* sp. (potentially two species). The species was particularly common in samples collected in early June 2000 at the Delaware Water Gap, PA, and at West Point Military Academy, NY while sites in the Fingerlakes Region and in Illinois showed a low occurrence of the species (Fig. 38). Attack rates of 20% of the stems, often with multiple larvae/stem were common (Fig. 38). Surprisingly, our data from 2001 show that the abundance of the species declined in the Northeast (Fig. 39) with the exception of a single site in the Hudson River Valley (Leon) where attack was also recorded in 2000 but at a much lower level (Fig. 38). This weevil occurs sporadically at many sites through the garlic mustard distribution and usually less than 10% of the stems show any signs of attack (Fig. 39).

We used data collected at field sites where this species was commonly encountered during 2000 to explore the relationship of habitat features and attack rates. Combining data from Westpoint, Watergap, and Leon showed that there was a weak relationship of the number of weevil larvae/0.25m^2 with the number of garlic mustard stems/0.25m^2 (Fig. 40). Weevil attack increased as the number of stems increased (Fig. 40). We further explored this relationship on a site-by-site basis (Figs. 41-44) and found that this relationship was particularly strong when weevils could be found in most of the stem samples (i.e. low occurrence of zero attack). Weevil larvae were often found aggregating in the lower third of the stem where much of the pith was mined when more than 3 larvae attacked a stem. Attacked plants did not show any signs of obvious damage and produced seed seemingly unaffected. Whether weevil attack does affect the quality or number of seeds will need to be determined under experimental conditions. Weevils complete their development in the stem, then exit the stem by chewing a hole in the stem and pupate in the soil. Adults emerge within a few weeks and feed on garlic mustard leaves. It appears that adults overwinter since adults can be observed feeding on garlic mustard leaves and flowers in early spring. Third instar larvae were frequently found attacked by an ectoparasitoid (we were unable to obtain adults for identification) paralyzing the mature larva. The ectoparasitoid larva develops, prepares a cocoon and overwinters in garlic mustard stems.

We collected adults of two *Ceutorhynchus* species (in the following called species 1 and species 2) at Dryden Lake in Central New York feeding in late April on leaves and flowers of garlic mustard. Species 1 appeared morphologically similar to the adults emerged from rearing of the stem-mining weevil. Adults of both species were sexed and two pairs were kept in transparent plastic cylinders (20cm high, 15cm in diameter) with cut shoots and developing seeds of garlic mustard. Cages were checked for feeding and stems were dissected in regular intervals. Adults of both species fed on garlic mustard leaves and stems. Feeding by adults of species 1created small randomly located shotholes on leaves but the majority of the feeding occurred on flower buds and leaf petioles. Feeding by species 2 was restricted to leaf margins but especially heavy on young stem parts. Species 1 had high survival rates and all adults, except for 1 were alive after 30 days, while mortality in species two was significant and no adults survived until day 30 of the experiment (Fig. 45). Females of species 1 laid eggs (although less than 1 egg/day) for two weeks after which oviposition collapsed. This was either due to the fact that the

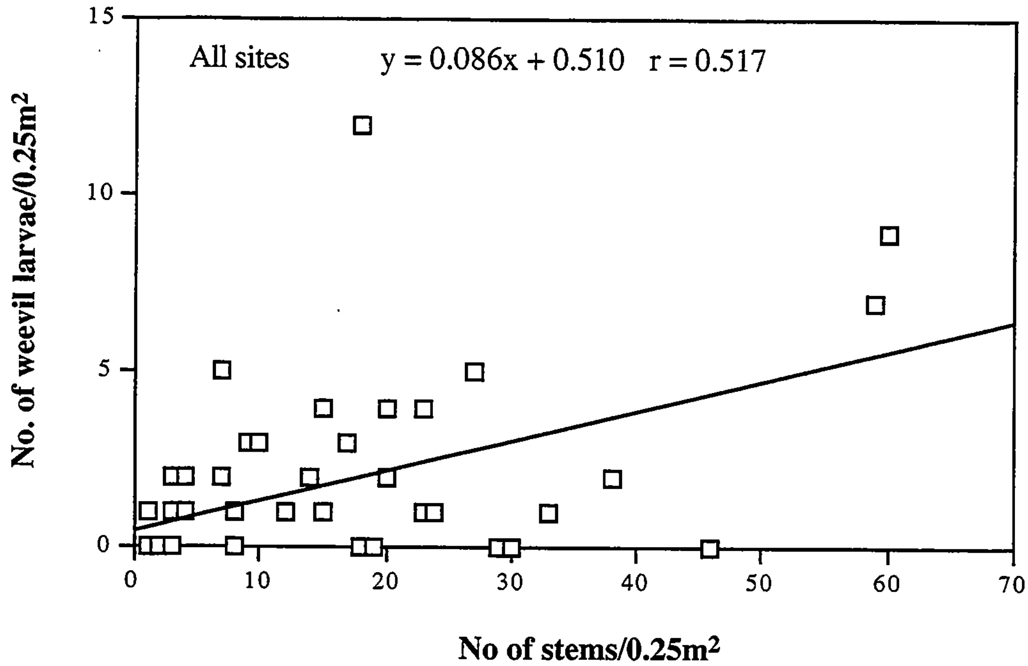

Fig. 40. Number of stem mining weevils/0.25m^2 as a function of the number of garlic mustard stems/0.25m^2. Data from 2000 dissections.

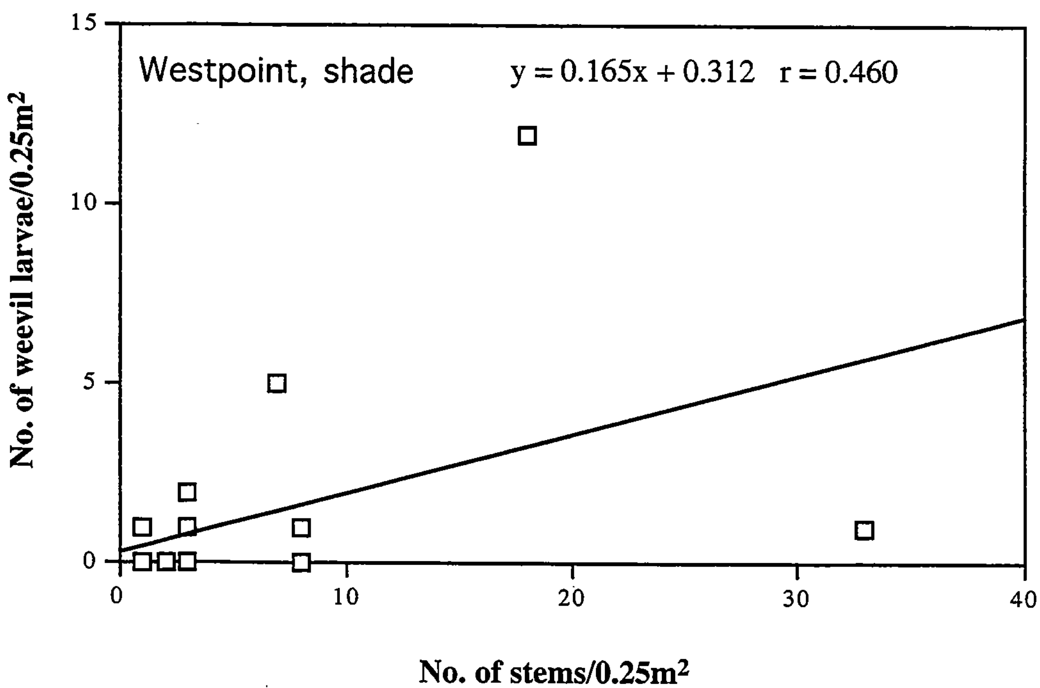

Fig. 41. Number of stem mining weevils/0.25m^2 as a function of the number of garlic mustard stems/0.25m^2 at Westpoint within the woods. Data from 2000 dissections.

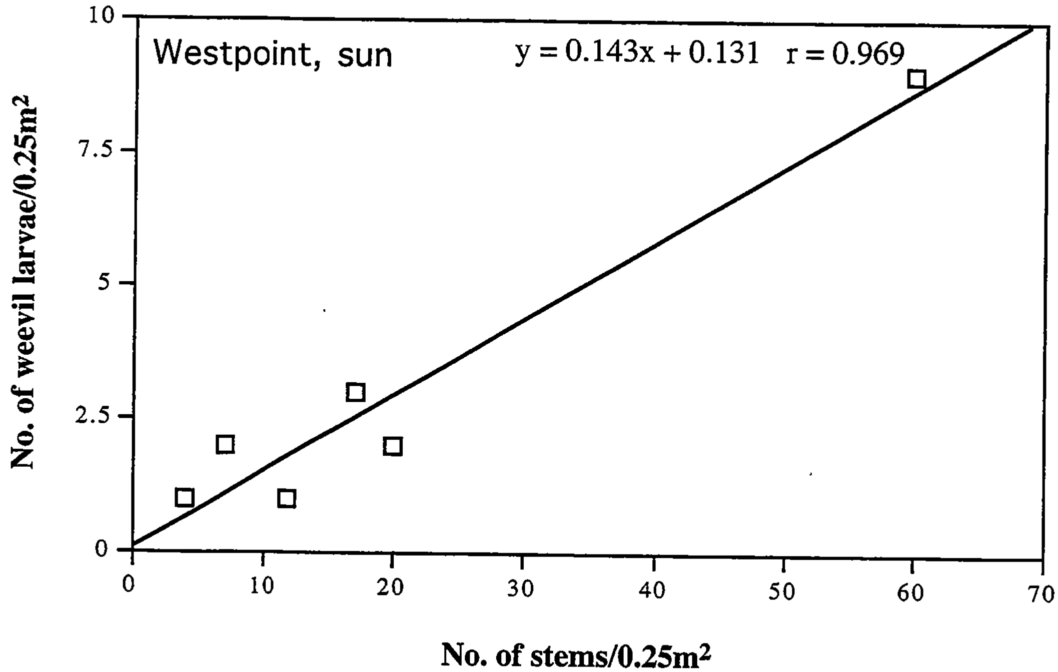

Fig. 42. Number of stem mining weevils/0.25m^2 as a function of the number of garlic mustard stems/0.25m^2 at Westpoint along the sunny woods edge. Data from 2000 dissections.

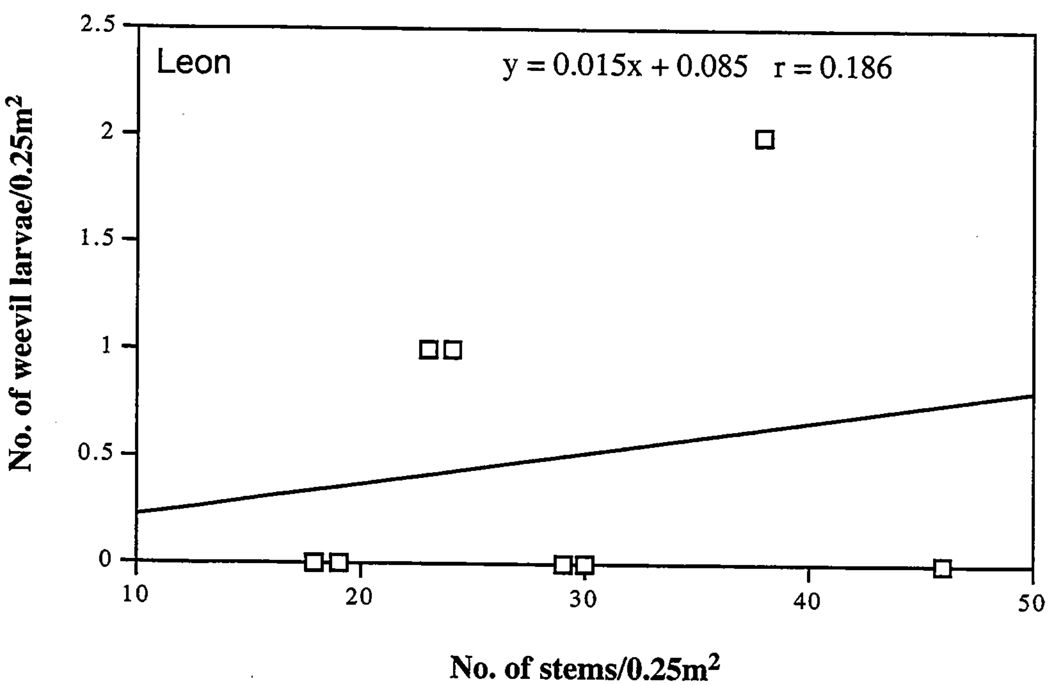

Fig. 43. Number of stem mining weevils/0.25m^2 as a function of the number of garlic mustard stems/0.25m^2 at Leon. Data from 2000 dissections.

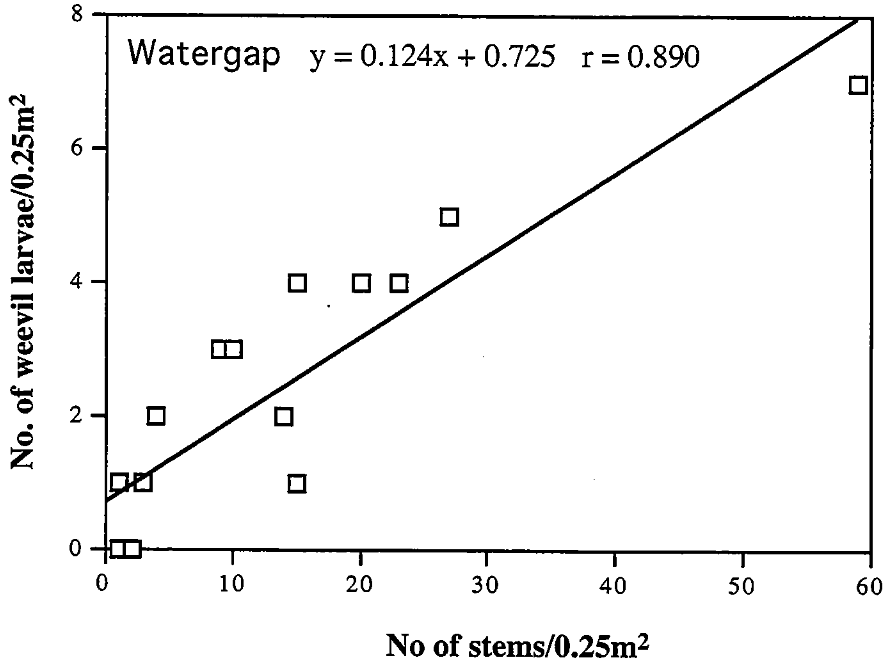

Fig. 44. Number of stem mining weevils/0.25m^2 as a function of the number of garlic mustard stems/0.25m^2 at the Delaware Watergap. Data from 2000 dissections.

natural oviposition for this species was over, or that exclusive feeding on garlic mustard does not allow continuous egg production. Eggs were laid into the middle of the stem, right below the developing siliques and larvae mined downwards. Feeding behavior of the larvae and damage pattern matched observations on the stem-mining weevil obtained during dissections. Larvae completed development in cut pieces of garlic mustard stems within 4 weeks. Eggs of species 2 were only observed during the very first oviposition period (Fig. 46). Eggs were glued to the outside of flower buds, no larval development occurred and we are uncertain whether the species can successfully develop on garlic mustard. As discussed for species 1, garlic mustard may not be the original (or primary) host of species 2. A tentative identification of species 2 indicated it to be a European introduction, *C. rapi*, a pest of Brassicaceae. However, this identification needs confirmation by a specialist. Adults of both species have been submitted to taxonomists but identification is still pending.

We occasionally observed a number of other species feeding on garlic mustard. Throughout the range, mirid true bugs could be found feeding on stems and root crowns; spittlebugs were common on stems and fruits, occasional heavy attack causing distortions of the infructescens. These species rarely reached attack levels above 1-3% of stems. In 2000 we observed a mass mortality caused by a fungus, *Alternaria* sp., of senescing plants at Leon in the Hudson River Valley. The fungus attacked 32% of the stems, causing a whitening of the stems with rice grain sized black fruiting bodies of the fungus developing inside the stem. The fungus often prevented seed set of attacked plants but did not affect rosettes or the return of a healthy garlic mustard population one year after the attack. We occasionally found the species at other field sites but attack rates were well below 1%. We also observed a stem scale outbreak at a single site in the Fingerlakes region (Leopold). The unidentified species caused stem distortions and swelling right below the developing siliques. At Leopold, 10% of all stems were attacked in 2000 and heavy attack prevented seed ripening. However, this was the only site we recorded this insect. Occasionally, larvae of a microlepidopteran species (identification pending) could be common on garlic mustard. Larvae appeared to be feeding preferentially in flowers but occasionally on leaves.

Overall, we found widespread but uncommon herbivory on garlic mustard throughout the range of the species in North America. With the exception of the leaf mining fly and of the stem mining weevil, we never found appreciable attack rates, and impact to garlic mustard was negligible. Considering that potential biological control agents investigated in Europe attack stems, seeds, and roots, there is little competition and many empty niches on garlic mustard in North America. While species identification for most organisms encountered is still pending, none of the potential biocontrol agents has been introduced to North America. Moreover, none of the herbivores presently attacking garlic mustard in North America has any potential as a biological control agent. The lack of herbivory in North America may have contributed greatly to the invasiveness of garlic mustard. Compared to the over 70 insects and fungi known from the native range, the herbivore community on garlic mustard in North America is not very diverse.

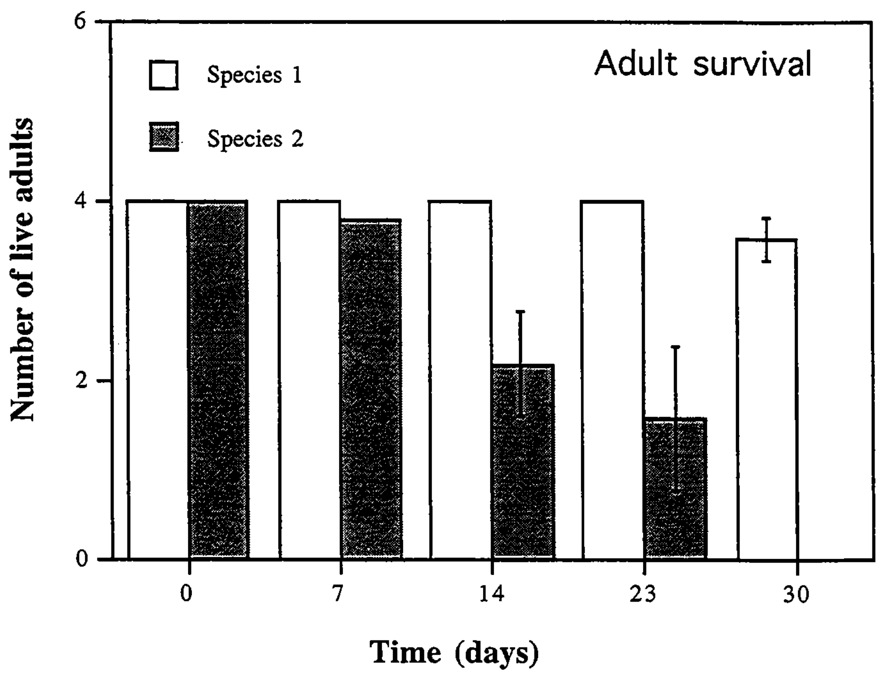

Fig. 45. Survival of two *Ceutorhynchus* species held on cut shoots of garlic mustard over a 30-day period. Data are means±SE of 5 replicates/species.

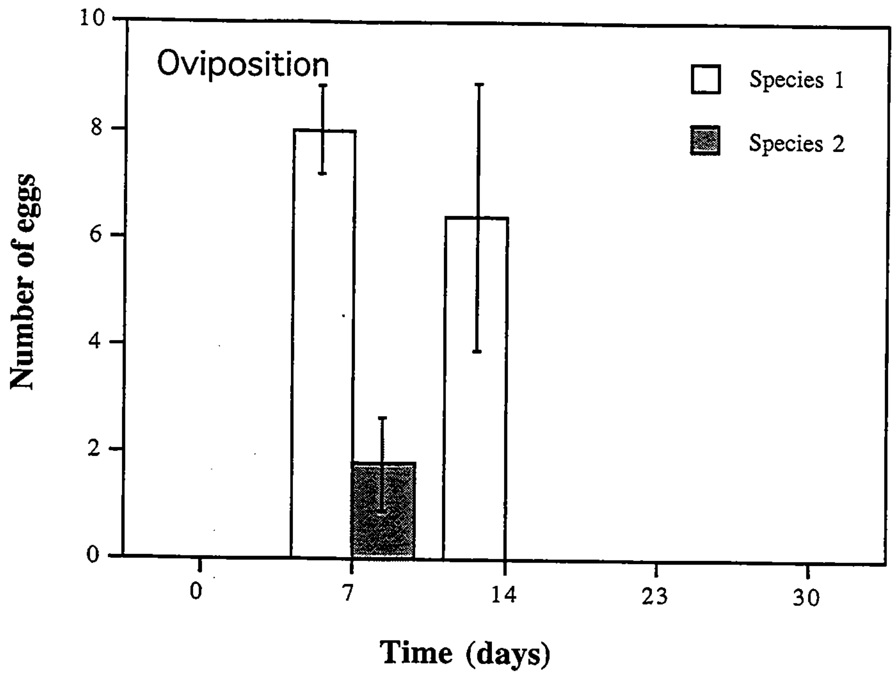

Fig. 46. Oviposition of two *Ceutorhynchus* species (number of eggs laid by 2 females/cage) on cut shoots of garlic mustard over a 30-day period. Data are means±SE of 5 replicates/species.

Seed predation of garlic mustard in North America

Introduction

Garlic mustard is an obligate biennial (i.e. plants die after their second growing season) and population persistence is entirely dependent on seed production. Plants produce an abundance of fairly sizeable seeds (3mm) in early summer and even very small plants are capable of producing a few seeds. Seeds are distributed by floods and most likely also transported by animals. Incipient populations are often observed at the base of trees, where small rodents may cache seeds or rest and groom themselves. Experiments in Europe demonstrated that rodents are a major seed predator for garlic mustard seeds in the native range. We were interested in investigating the role of vertebrate and invertebrate seed predators in North America. The goal of the biocontrol program is ultimately to reduce seed production, which should lead to reductions in population densities. The presence of, and seed removal by, native North American seed predators may aid in reducing the garlic mustard seed bank. Garlic mustard can commonly be found along edges of roads and woods in dappled light but the species is now also found in full sun and also in full shade. We established seed removal experiments in different habitats using cages with different mesh sizes to allow access by differently sized seed predators. We also established experiments in areas with, and in areas without, an existing garlic mustard population to assess the response of the biotic community to a common or "novel" food source.

Methods

Exclosure cages (30 x 30 x 30cm) were manufactured using 2.5 x 2.5cm wood to create a frame. We designed three different types of cages; (1) exclude all seed predators (control); (2) access allowed for invertebrates but exclude vertebrate predators (mice excluded); and (3) allow access by all seed predators (open access). All cages were fitted with metal window screen at the bottom to prevent access to seeds from below the cage. Each cage was also fitted with a screw-on lid built out of wood strips and metal window screen to prevent access by predators from above. Using additional 1cm thick and 2cm wide foam insulation strips between cage and lid enabled a tight seal when the lid was screwed onto the cage frame. Metal window screen was stapled to all sides for cages designed to exclude access by all seed predators. Wire mesh (opening size approx. 1 x 1cm) was stapled to all sides on cages designed to exclude vertebrate predators. This mesh size allowed potential invertebrate predators (slugs, earthworms, beetles) to access seeds. All sides (except for the bottom and lid) were left open for cages designed to allow access by all seed predators.

We established the experiment at two locations (Leopold, REM) in Ithaca, NY in late May 2001. Leopold is a southwesterly facing wooded slope along the shores of Cayuga Lake with an abundant understory of garlic mustard. Plants grow in discrete patches throughout the wooded area. REM is a riparian area along the shores of Cascadilla Creek crossing the Cornell Campus. Open (occasionally mowed) small meadows mix with brush (mostly *Lonicera* spp.) under a tree canopy. At REM, garlic mustard is found along edges of brush, largely in small clumps or as individual plants. At each site we randomly located 30 cages as outlined in Table 19.

Table 19. Treatments, number of replicates, and site location information for garlic mustard seed predator experiment.

Treatment/Site	Leopold		REM	
	A. petiolata present	*A. petiolata* absent	*A. petiolata* present, brush	*A. petiolata* absent, meadow
Open access	5	5	5	5
Mice excluded	5	5	5	5
All excluded	5	5	5	5

We first located sites for all treatments by placing a numbered flag into an appropriate area. Distance between cage locations at Leopold was at least 10m and 2-5m at REM. Five replicates for each cage design were then assigned to locations in each of the four areas by random drawing. Cages were established in the field by removing a 2cm deep layer of the soil to create level ground at each location. The cage was then put in place and washed sand was poured onto the bottom until it was level with the surrounding soil (a layer of approx. 2.5cm). The weight of the sand secured the cage at each location and the homogeneity of the sand allowed easy recovery of seeds. The soil removed from under each cage was then used to smooth out any height differences between the cage interior and the outside soil. We placed a 10 x 20cm piece of scotch-brite foam level with the sand into the center of each cage. We then carefully scattered 100 garlic mustard seeds onto the foam, placed and secured the lid. We checked cages at 14-day intervals from June-November 2001 when snow cover prevented the continuation of the experiment. This encompassed a period from before seed set of garlic mustard (beginning in late June) to winter. Each time cages were checked, the screw-on lid was removed from all cages (including the open-access cages), the foam with remaining seeds was placed into a plastic bag and the sand was carefully examined for scattered seeds that may have fallen off the foam. All seeds encountered were placed into a labeled plastic bag and returned to the laboratory for examination. We placed a new foam into the center of the cage and scattered another 100 garlic mustard seeds onto it. At each visit, sand in the cage and the surrounding soil were leveled to remove height differences and allow easy access by the (allowed) seed predators. In the lab, remaining seeds were counted under a stereomicroscope and notes were taken on seeds that showed feeding damage.

We also established automatic cameras for 3 weeks in July 2002 to record potential vertebrate activity at our seed exposure cages.

Results

Our seed exposure and recovery methods worked extremely well and seed recovery rates from control cages were 90% or higher at all sites (Figs. 47-50). This initial seed loss is likely explained by handling or due to rain washing seeds off the pads. As the season progressed, seed loss in control cages increased at all sites to near 20% and variability increased. We attribute this to an increase in severe weather in the fall with rainstorms and high winds. After leaf fall, seeds in cages are exposed significantly more to the elements than when protected by a leaf canopy. Mice or other rodents were the most significant seed predators of garlic mustard and seed removal by invertebrates contributed little to overall seed loss (Figs. 47-50). Seed removal patterns differed strongly between Leopold and the REM and also in patches with and without presence of garlic mustard. We exposed seeds before seed set by garlic mustard, yet seed removal at Leopold was 50% after the first two weeks in both the garlic mustard and non garlic mustard patches (Figs 47, 48). The second control saw a dramatic shift in seed removal with a drop in the non garlic mustard patches while seed removal in garlic mustard reached 80% and stayed high for several weeks (Fig. 47). It is likely that coincident with seed set in garlic mustard, mice were moving into garlic mustard patches and consuming a significant portion of the exposed seeds. Over time, seed removal rates became more similar and by the end of the experiment were nearly identical regardless of presence or absence of garlic mustard. Invertebrate predation at Leopold was insignificant with 5-10% of seeds removed in early summer. After August, seed loss in control cages and in cages where mice were excluded was no longer different from each other (Figs. 47, 48).

At REM, invertebrate predation in the meadow and in brush was higher than at Leopold. In July and August, invertebrate seed predation accounted for all seed removal in the open site, while mice were always more important in brush (Figs. 49, 50). We did not find a rapid increase of mouse predation in garlic mustard patches at REM, but rather a steady increase and then stabilization in seed removal rates (Fig. 49). In the fall, seed removal by rodents at REM from cages established in the vicinity of garlic mustard was higher than seed removal at Leopold but similar to seed removal by rodents at the meadow site. Overall, the seed removal at Leopold showed large seasonal and site-specific fluctuations while seed removal was more stable at the REM.

The identity of invertebrate predators remains unresolved. We found earthworms, slugs and beetles active in and around our cages but we are uncertain which species may be responsible for seed removal. In a related project studying the ground beetle community at Leopold, all carabids captured in pitfall traps established at the site were carnivorous predators and no known seed predators were encountered. Our automatic camera established at REM seed exposure cages captured abundant white footed mouse (*Peromyscus leucopus*) activity. It appears that this species is the major seed predator of garlic mustard at our field sites. A number of seeds recovered at each check showed mouse feeding damage and occasionally sections of the seed were removed. This additional granivory due to unknown organisms contributed little to seed loss.

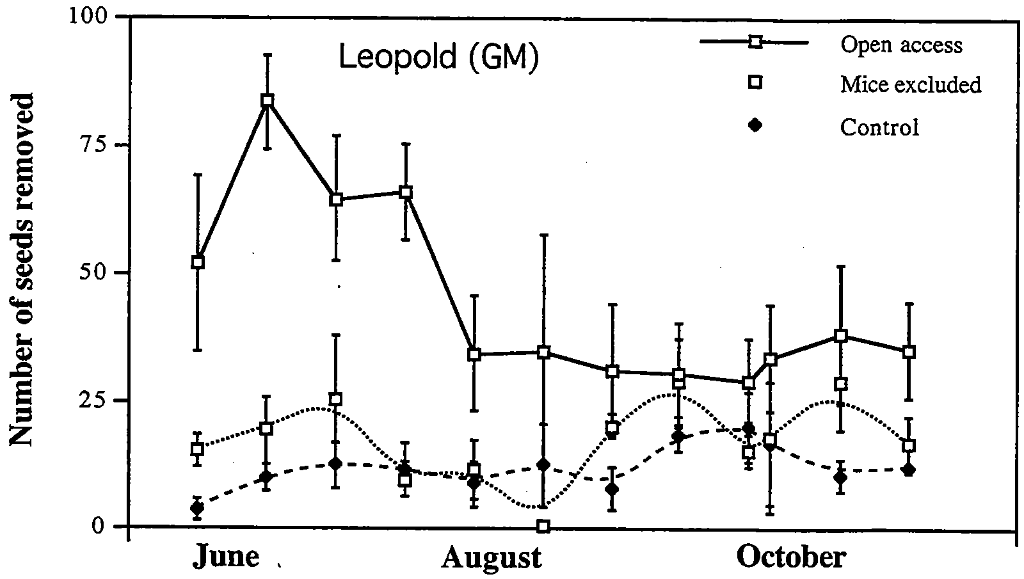

Fig. 47. Number of garlic mustard seeds removed in 14-day intervals during the 2001 field season from cages established in existing garlic mustard patches at Leopold, Ithaca, NY. Solid lines represent cages allowing free access by seed predators, stippled lines represent cages where mice were excluded and dashed lines represent control (access by seed predators prevented). Data are means ±SE of 5 replicates per treatment.

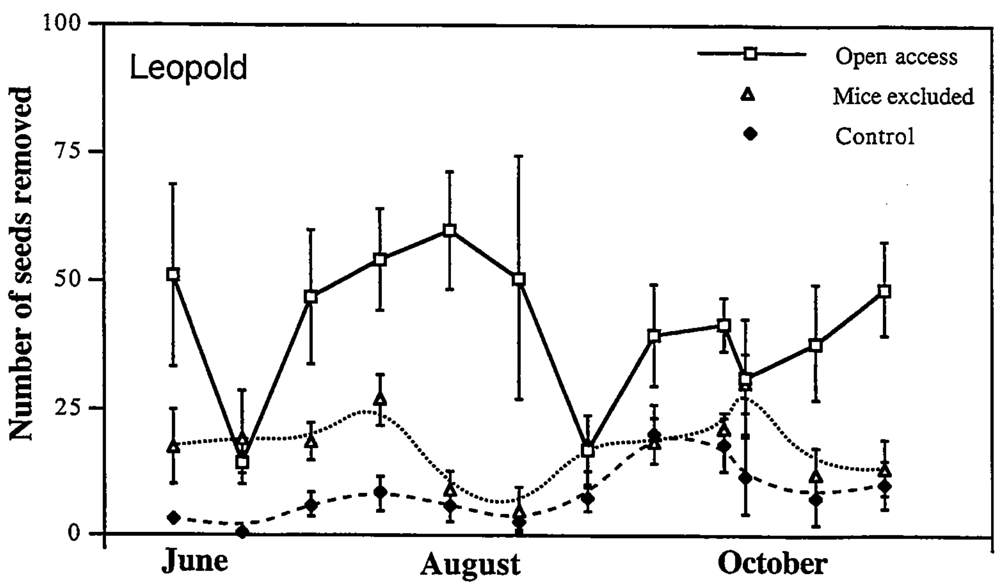

Fig. 48. Number of garlic mustard seeds removed in 14-day intervals during the 2001 field season from cages established in garlic mustard free areas at Leopold, Ithaca, NY. Solid lines represent cages allowing free access by seed predators, stippled lines represent cages where mice were excluded and dashed lines represent control (access by seed predators prevented). Data are means ±SE of 5 replicates per treatment.

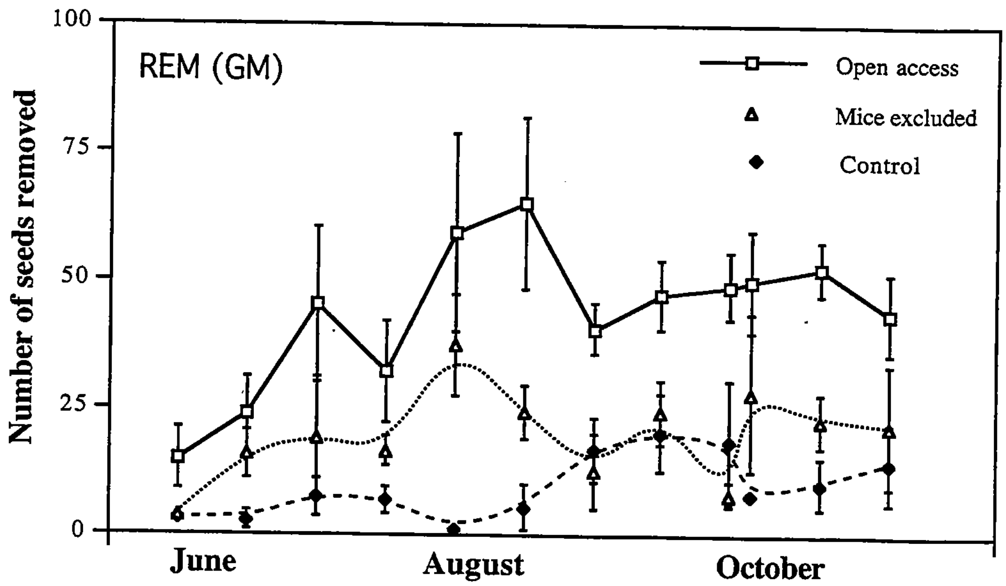

Fig. 49. Number of garlic mustard seeds removed in 14-day intervals during the 2001 field season from cages established in existing garlic mustard patches at REM, Ithaca, NY. Solid lines represent cages allowing free access by seed predators, stippled lines represent cages where mice were excluded and dashed lines represent control (access by seed predators prevented). Data are means ±SE of 5 replicates per treatment.

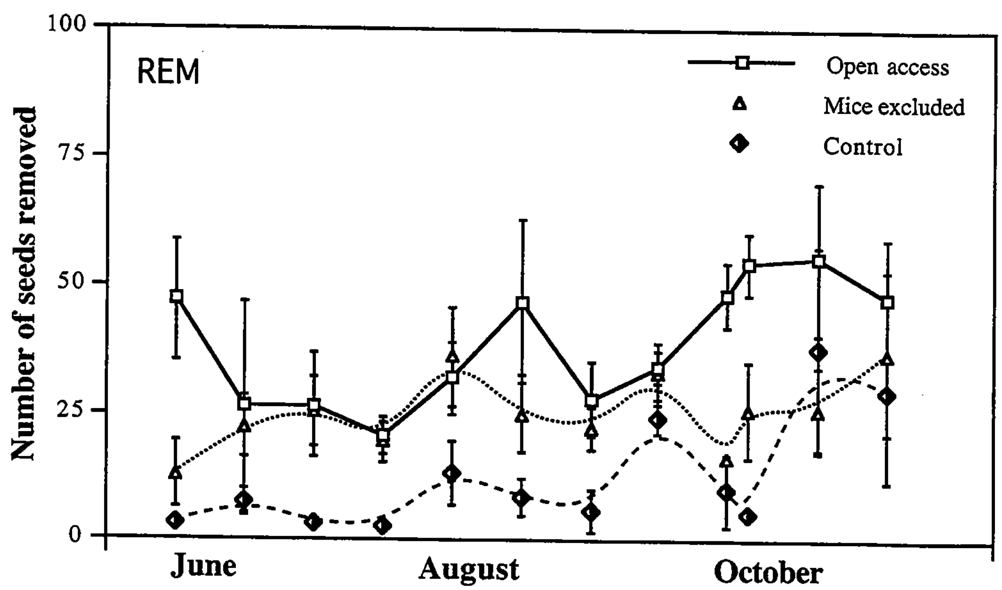

Fig. 50. Number of garlic mustard seeds removed in 14-day intervals during the 2001 field season from cages established in an open meadow at REM, Ithaca, NY. Solid lines represent cages allowing free access by seed predators, stippled lines represent cages where mice were excluded and dashed lines represent control (access by seed predators prevented). Data are means ±SE of 5 replicates per treatment.

Overall, we were surprised by the intensity of vertebrate and invertebrate seed predation on garlic mustard. Regardless of habitat type, seed removal in two weeks amounted to nearly 50% throughout much of the season. The tendency of white footed mice to cache food may contribute to dispersal of garlic mustard. Home ranges of this species are quite large (up to 0.5 ha) and animals travel significant distances. This may contribute to the observed "sudden" occurrence of discrete patches of garlic mustard away from the invasion front (Nuzzo 1993, 1999). Regardless of habitat, North American seed predators remove a significant portion of seed of this introduced species annually. However, this seed predation was obviously unable to prevent the spread of garlic mustard through much of North America.

DEVELOPING A MONITORING PROTOCOL FOR GARLIC MUSTARD

(Alliaria petiolata)

Introduction

Quantitative monitoring of the biocontrol insect, target plant, and associated plant community is an integral part of a natural areas biocontrol program (Blossey 1999). Monitoring, defined here as the collection and analysis of repeated measurements, permits identification and evaluation of changes over time, and allows the ability to make inferences about cause and effect of observed changes. An apparent decline in a target plant population may be due to the biocontrol agent, or may be related to climate fluctuations, attack by other insect species, or other factors. Data obtained through long-term monitoring allows investigators to quantify the abundance and impact of the biocontrol agents, the response of the target plant to the agents, and the response of the community to these changes. These combined factors can then be used to evaluate 'success' of the biocontrol program, compare predicted to actual impacts, and gain insight into how ecosystems respond to changes in abundance of individual species (Blossey 1999).

Monitoring protocols are based on the target plant's biology and the biocontrol insect's biology, and are developed within a real-world context. For garlic mustard (and other natural area biocontrol targets) this includes use by personnel with widely varying skill levels, in sites of widely varying characteristics. Measures need to be robust enough to reliably assess biocontrol impact, accommodate different personnel and skill levels, and still yield statistically useful data. Data collection should be labor efficient (for example, one-half day/site/year), simple, easy to use, and low cost. While a complex and detailed protocol can yield in-depth data, it is less likely to be used, and thus less useful on a regional or national basis, than a simpler protocol.

A standardized monitoring protocol allows widespread participation in assessing the impact of control agent release across North America. Standardization also allows comparison of data collected by participants in different locations across the country.

Methods

Garlic mustard is an obligate biennial (all plants die within two years), and can only spread by seeds; therefore the goal of biocontrol is population reduction, achieved through reduction in total seed production. Seed production in turn is reduced by direct plant mortality before flowers or seeds are formed, and/or by reduced plant vigor resulting in fewer seeds and/or fewer siliques.

Garlic mustard seeds germinate in early spring, and form a basal rosette by June. Plants remain as rosettes through the winter, and produce flower stalks the following spring, usually blooming in April - May, and producing seeds in siliques (linear pods) 4-8 weeks later, usually in June-July. Individual stems produce an average of 350 seeds, varying from 0 to >7,000 (Nuzzo 1999) and seeds live 3-5 years in the seedbank. Seeds require cold stratification (~ 100 days at 2-5° C) in order to germinate. Depending on climatic factors, this is usually achieved within one to two years. Seedling population size in any one year is influenced by seed production in prior years, seedbank survival, and winter temperatures. One or both age classes (seedling and adult) may be present in any given year and site. Each individual lives approximately 16 months (germinating in April of year 1 and dead by August of year 2). Population density is highly variable year to year (Nuzzo 1999): A decline in any one year may be due to natural fluctuations, and/or biocontrol impact.

Four weevil species affecting seeds, stems, and roots are under study for introduction as biocontrol agents. These weevils appear to impact garlic mustard by: 1) causing direct mortality (adult feeding on seedlings, and larval and/or adult feeding on leaves and root crowns; 2) reducing plant vigor, resulting in fewer resources being available for seed production; and 3) direct seed predation.

Adults of all four weevil species are small, blackish, and are difficult to observe directly, as they 'blend in' on their host plant, and drop easily off the plant when disturbed. Only adults feed externally on leaves, creating a characteristic 'windowpane' feeding pattern that can be easily recognized. In addition, adults feed on stems and petioles, leaving a 'scraping' mark. Eggs are laid into plant tissue and larvae feed internally on seeds, stems, and rootcrowns. Larvae induce most of the damage, but because they feed inside the plant they are not usually observed. Under heavy attack by one or more of the weevil species, garlic mustard plants become shorter, can produce more but thinner stems, are less robust, often have tip dieback, and produce fewer siliques (although inflorescence number can increase).

Because weevil species presence and abundance cannot be accurately determined in the field through direct counts, presence of weevils is usually based on finding 'windowpane' feeding patterns. The impact of biocontrol agents on plant performance will be difficult to assess directly. Insect-induced mortality will be difficult to separate from other sources of mortality. Garlic mustard populations naturally undergo wide fluctuations in density and cover from year to year (Nuzzo 1999). Determining impact of weevils, versus other factors, therefore needs to be based on dramatic reduction in populations and annual seed production, and persistent reduction in garlic mustard stem density over time.

Monitoring Biocontrol Impact on Garlic Mustard

The desired outcome of biocontrol is a dramatic reduction in abundance of garlic mustard. Therefore, at a minimum, a monitoring protocol should be capable of 1) Detecting presence, and measuring abundance of biocontrol agents; 2) Detecting changes in garlic mustard plant performance and seed production; 3) Detecting change in garlic mustard population trends; 4) Allowing correlation of weevil abundance to change in garlic mustard abundance; and 5) Detecting change in groundlayer plant communities.

Destructive sampling yields accurate data for weevil presence and impact, but kills both plants and weevils and is therefore not an appropriate method. In addition, destructive sampling is time-consuming, resulting in fewer samples and less data overall. In light of this, and the biology of the weevils and garlic mustard, general guidelines were established for developing and testing a garlic mustard monitoring protocol:

1. Document garlic mustard growth (stem height, stem diameter, silique and seed production) and insect attack in Europe, using field collections and destructive sampling.
2. Document garlic mustard growth and insect attack in the US prior to introduction of biocontrol, by:
   a. Destructive sampling of field collections from multiple locations across the range of garlic mustard; and
   b. Establishing permanent sampling sites and annually recording multiple measures of garlic mustard growth and insect attack, and community composition.
3. Analyze and compare all data sets to detect trends, correlations, and predictive variables.
4. Determine which non-destructive measures provide most useful, robust, and reliable data.
5. Develop, field-test, and refine a monitoring protocol based on these non-destructive measures.

Data Collection

We selected four permanent study sites in different areas of North America (Fermilab and Hall Woods in IL, Leopold and West Point in NY, Table 20) for long-term monitoring and to test preliminary versions of the monitoring protocol. At each site garlic mustard was well established, with both age classes present. Prior data was available for three of the four sites (Table 20). We initially planned to establish permanent quadrats at Ft. Drum, NY, but detailed field visits revealed that garlic mustard densities were too low and sites were also invaded by *Lonicera* spp. (bush honeysuckles) making these sites unsuitable for long-term monitoring.

Permanent plots were established at all sites in May 2000. At three sites (Fermilab, Hall Woods and West Point) 24-26 $0.5m^2$ (1.0m x 0.5m) permanent quadrats were established along two parallel transects, with the first quadrat randomly established and subsequent quadrats located at 10m intervals. Transects were located 5-25m apart, beginning near the forest edge and extending into the forest interior. At the fourth site (Leopold) $0.5\ m^2$ quadrats were established in an irregular pattern, 13 in 2000, and an additional 11 in 2001. Initial quadrat locations occasionally needed to be adjusted to ensure garlic mustard presence; thus, permanent quadrats likely overestimated actual mean garlic mustard density in the first year, but thereafter are assumed to provide an accurate estimate of garlic mustard density.

Table 20. Location, size, and characteristics of permanent study sites.

	Fermilab	Hall Woods	Leopold	West Point
Location	Batavia	Rockford	Ithaca	
State	IL	IL	NY	NY
Owner	US-DOE	Rockford Park District	Private	US-DOA
Size	32 ha	16 ha	4 ha	>50 ha
Forest Type	Mesic Upland	Dry-mesic Upland	Dry-Mesic Upland	Mesic Upland
Soil	deep loamy clay	deep loam	thin sandy loam over shale	thin loam over shale
Slope	0-2%	0-2%	5-10%	5-10%
Aspect	na	Na	West	Northwest
# Quadrats	24	24	24	26
Existing data	Community-wide: Cover, frequency at 2 year intervals, 1992 -2002	Community-wide: Cover, frequency, and adult density, 1989-1992, 1997	Individual plants: Demography, 1996-1997	None

Permanent quadrats were used in favor of rerandomized quadrats for several reasons: to follow each generation of plants from seedling to adult; to have statistically strong data with lower time input (fewer permanent quadrats provide similar statistical strength as many more rerandomized quadrats); to increase time efficiency (once established, relocating permanent quadrats is more time efficient than establishing new random quadrats); to reduce the chance for investigator bias in locating quadrats each year (for example, by placing quadrats in areas of 'high' or 'low' density); to maintain consistency year to year regardless of change in investigators; and to assess change in associated community (which is composed of many perennials, and permanent quadrats allow a better assessment of community change).

Quadrat size ($0.5m^2$) and number (24) were selected to capture spatial and temporal variability of garlic mustard. Once established, garlic mustard remains present in subsequent years in 95-100% of infested quadrats but density can vary from very low to very high, in one or both age classes (Nuzzo 1999, Meekins 2000). Within each quadrat, density data were recorded independently within each half of the quadrat. This allowed assessment of the predictability of two differently sized sampling quadrats ($0.25m^2$ and $0.5m^2$).

Data were recorded for both *Alliaria* and other members of the community in June and October 2000-2002 in the permanent quadrats to coincide with seed production and rosette development, respectively. Data recorded included measures of garlic mustard performance and community composition (Table 21). Cover was estimated within cover classes (present, <1%, 1-5%, and in 10% increments thereafter). Litter depth was measured to the nearest cm in the center of each half of the $0.5m^2$ quadrat.

Table 21. Data recorded from permanent quadrats in June and October, 2000-2002.

	June	October
Garlic Mustard:	Percent cover all garlic mustard	Percent cover rosettes
	Percent cover adults	
	Percent cover seedlings	
	Density of adult stems	Density rosettes
	Height of Adult stems	
	Number of siliques/stem	
	Density of seedlings (2001 – 2002)	
	Presence and amount of external	Presence and amount of external
Community:	Percent cover by species	Percent cover by species
	Percent cover soil, leaf, wood, rock	Percent cover soil, leaf, wood, rock
	Litter depth (cm)	Litter depth (cm)

Analysis of the 2000 data provided insight into accuracy and needs of the preliminary monitoring protocol. Several concerns were apparent:

1. Rosette density was very difficult to count accurately, particularly when rosettes were very dense and/or small;
2. Biomass provided the most accurate assessment of garlic mustard abundance, but biomass could not be collected in permanent quadrats;
3. Background levels of insect herbivory were needed to accurately assess the additional herbivory experienced by biocontrol insects after their release; and
4. Garlic mustard density and presence by size class fluctuated widely from year to year, and from season to season due to the species' biennial nature, overlapping generations, and weather-induced mortality. This was most noticeable at West Point in 2001, where seedlings were present in all 26 quadrats in June, and rosettes occupied only one quadrat in October (Table 24).

Therefore, additional data were recorded beginning in 2001. Counting seedlings and rosettes was time-consuming and often difficult, particularly when plants were very small or densely crowded, and/or at high density or obscured by newly fallen leaves; thus, rosette density was first estimated within four classes (1-10, 11-25, 26-1090, 101-500) and then counted. Estimated density was then compared to actual density. We found that estimated density inflated garlic mustard abundance at the highest abundance class (actual density in October 2001 was always <150 plants, considerably lower than the 300 plants assumed by estimation). Density was underestimated in the two lowest classes, because small rosettes were either invisible under leaves, or clusters of small rosettes appeared to be single rosettes. This method was therefore discarded, and actual density counts were continued despite the acknowledged difficulties.

Rosette size affects survival and flower and seed production (unpublished data), and two measures of rosette size were tested. In 2000 diameter of individual rosettes was measured to the nearest mm at one site. This method proved impractical due to high time costs and potential damage to small rosettes. In 2001 'average' rosette size/quadrat was estimated within four diameter classes (<2cm, 2-5 cm, >5-10cm, >10-15cm). This method proved impractical, as rosette size often was highly variable within quadrats.

Presence of external attack was recorded to document 'background' levels of herbivory prior to introduction of species-specific herbivores. Many methods were tested and several discarded; the selected method consisted of 1) recording presence/absence of easily recognized insects (spittlebug and scale); insect herbivory (leaf mines, windowpane feeding, edge feeding, and holes), deer browse, and disease; and 2) estimating percent of total leaf area removed by insect attack within "percent removed" classes (06-25%, 26-50%, 51-75%, 75-95%, and > 95%). This method has limitations but is the most useful method to approximate intensity of insect attack. Overestimating attack is rare with this method; rather, there is potential to underestimate feeding attack when individual leaves are completely removed, or plants are completely defoliated, and thus neither detected nor included in the 'percent removed'.

At one site (Leopold), data were also recorded in April and December 2001 to investigate optimal time period to monitor, and to assess benefit of monitoring more than twice each year. While seedling cover was higher in April than June (reflecting intraspecific mortality) and rosette cover was slightly higher in December than October (reflecting cool-season growth)(Table 22), data recorded in June and October provided greater information than data recorded in April and December. The additional data was deemed insufficient to justify monitoring three or four times a year. Therefore, monitoring was restricted to June and October.

Table 22 : Leopold 2001. Repeated sampling of permanent quadrats

	April	June	October	December
Number quadrats	13	22	24	24
Total percent cover	35.4	49.3		
Adult cover	23.2	42.5		
Seedling cover	12.1	8.4		
Rosette cover			2.8	3.4
Mean Density/m2			24.1	15.7
Estimated Density/m2			30.0	15.1
Estimated mean diameter (cm)			1.8	2.1

Data were compiled, analyzed, and evaluated for accuracy and efficiency in collection, and preliminary monitoring protocols were developed and tested each year. In June of 2002, 30 land managers participated in a workshop to test the draft monitoring protocol, and their suggestions were incorporated into the final draft Monitoring Protocol.

RESULTS

Garlic mustard was present in every quadrat of every site in June (with one exception) (Table 23). Presence in October was almost always much lower than in June.

Table 23. Frequency of garlic mustard in June (adults and seedlings combined) and October (rosettes) in all study sites, 2000 – 2002.

		Fermi	Hall	Leopold	West
2000	June	100%	100%	100%	100%
	October	92%	54%	100%	96%
2001	June	100%	100%	100%	100%
	October	71%	90%	71%	39%
2002	June	100%	100%	100%	96%
	October	100%	33%	88%	77%

Frequency by age class varied among sites and years; seedling frequency was consistently the highest, and adult frequency the lowest, reflecting natural mortality through time (Table 24). At all sites a generation of high adult frequency alternated with a generation of lower frequency.

Table 24. Frequency of garlic mustard by age class at all study sites, 2000 – 2002.

		Fermi	Hall	Leopold	West
Generation 1					
June 2000	Seedling	96.00%	92.00%	100.00%	100.00%
October 2000	Rosette	92.00%	54.00%	100.00%	96.00%
June 2001	Adult	88.00%	46.00%	100.00%	96.00%
Generation 2					
June 2001	Seedling	79.00%	96.00%	82.00%	100.00%
October 2001	Rosette	71.00%	91.00%	71.00%	4.00%
June 2001	Adult	54.00%	80.00%	50.00%	12.00%
Generation 3					
June 2002	Seedling	100.00%	85.00%	100.00%	96.00%
October 2002	Rosette	100.00%	33.00%	88.00%	77.00%

Garlic mustard cover, density, height, and silique production varied significantly throughout the study period, both within and between sites. Percent cover varied by 800% between years (Figures 51-53) and both stem and rosette density fluctuated 500% between years (Figures 54-55). The vast majority of stems were fertile, but sterile stems were present at all sites in all years, usually <5% of total stem density, but occasionally as high as 24%. Stem height varied between years, but less so than other measures of abundance (Figure 56). Silique production also varied significantly, both on a per stem basis (Figure 57) and on a quadrat basis (Figure 58).

Silique production/quadrat was strongly and positively correlated with percent cover (Figure 59) ($p<0.001$, $r^2 = 0.75$) and density ($p<0.001$, $r^2 = 0.40$) of adult garlic mustard. While adult cover and adult density were strongly correlated ($p<0.001$, $r^2=0.54$), adult cover explained the majority (69%) of the variation in silique production/quadrat (Table 25); density and height provided just 0-4% additional explanation). However, stem height explained the majority (75%) of the variation in silique production/stem (Table 26). Neither measure of silique production (/stem or /quadrat) was correlated to stem density (Figure 60).

Table 25. Multiple regression model of (A) Adult cover, (B) Adult density, and (C) Mean stem height, against total silique production/0.5m^2 (all sites and years combined).

P	Cp	Adjusted R Square	R Square	Resid Ss	Model Variables
1	534.2	0.0000	0.0000	7348357	Intercept only
2	40.4	**0.6916**	0.6932	2254133	**A (Adult cover)**
2	325.9	0.2902	0.2941	5187279	B
2	411.5	0.1698	0.1744	6066780	C
3	12.4	0.7323	0.7352	1945818	A C
3	42.3	0.6900	0.6934	2253252	A B
3	110.9	0.5929	0.5974	2958456	B C
4	4.0	0.7455	0.7497	1839105	A B C

Table 26. Multiple regression model of (A) Adult cover, (B) Adult density, and (C) Mean stem height, against mean silique production/stem (all sites and years combined).

P	Cp	Adjusted R Square	R Square	Resid Ss	Model Variables
1	536.9	0.0000	0.0000	17240.0	Intercept only
2	7.0	**0.7394**	0.7409	4467.37	**C (Mean Height)**
2	483.6	0.0720	0.0771	15911.6	B
2	525.7	0.0130	0.0184	16922.9	A
3	2.5	0.7472	0.7499	4310.86	A C
3	3.2	0.7462	0.7490	4327.64	B C
3	355.7	0.2498	0.2580	12791.7	A B
4	4.0	0.7465	0.7507	4298.64	A B C

Adult abundance (cover, density) and silique production showed little correlation to seedling cover in either of the following two years (Figure 61). Thus, these factors do not function as predictors of future seedling abundance.

Rosette density in October was strongly and positively correlated with adult density the following June (Figure 62). This is the stage most sensitive to attack by the proposed biocontrol weevil *Ceutorhynchus scrobicollis*.

Leaf attack was recorded at all sites and in all years, ranging from <1% to ~15% of leaves removed (Figure 63). This represents background herbivory and disease.

Community measures (mean number of species and mean percent cover) fluctuated nonsignificantly between years (Figures 64-65) with one exception (Fermilab 2002). This large increase in cover was due to an unplanned fire that stimulated growth of native vegetation. Thus, a significant and consistent change in these measures following introduction of biocontrol would indicate a community response to the predicted reduction in garlic mustard.

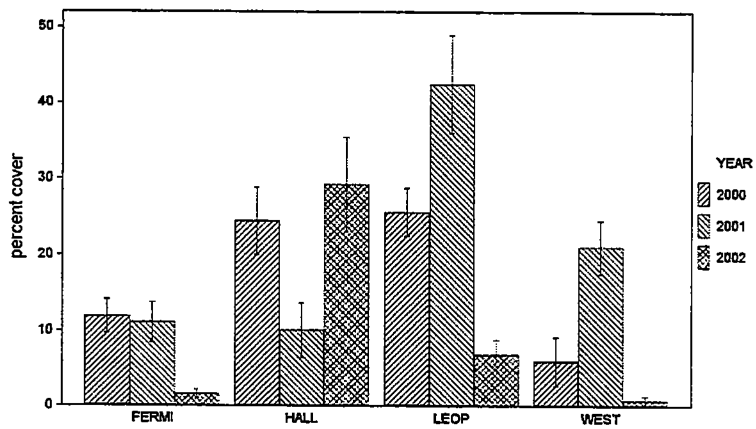

Fig. 51. Percent cover of adult garlic mustard in June at all sites, 2000 – 2002.

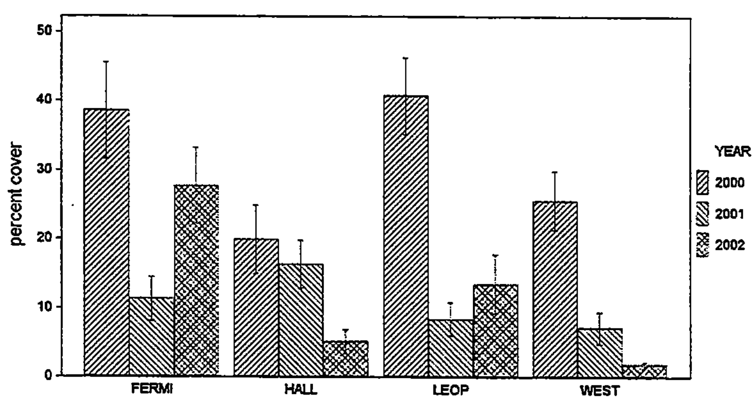

Fig. 52. Percent cover of seedling garlic mustard in June at all sites, 2000 - 2002.

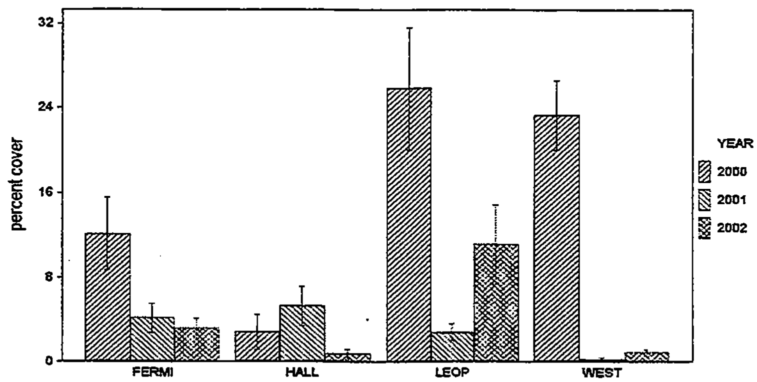

Fig. 53. Percent cover of rosette garlic mustard in October at all sites, 2000 - 2002.

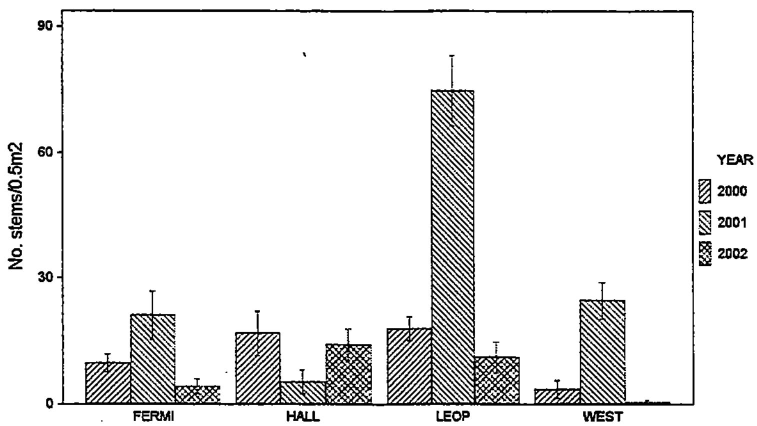

Fig. 54. Garlic mustard mean stem density/0.5m^2 in June at all sites, 2000 - 2002.

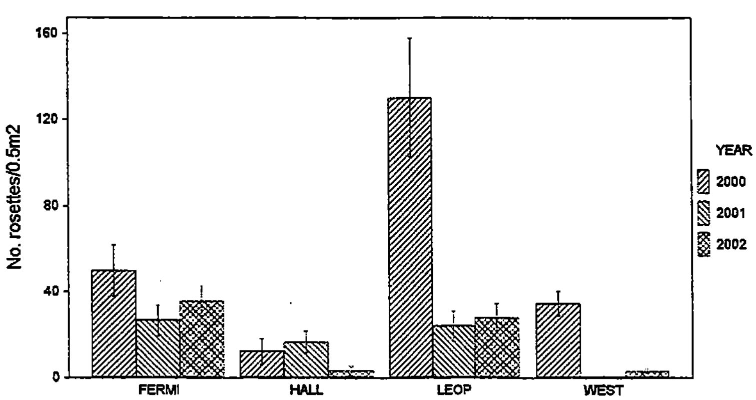

Fig. 55. Garlic mustard rosette mean density/0.5m^2 in October at all sites, 2000 - 2002.

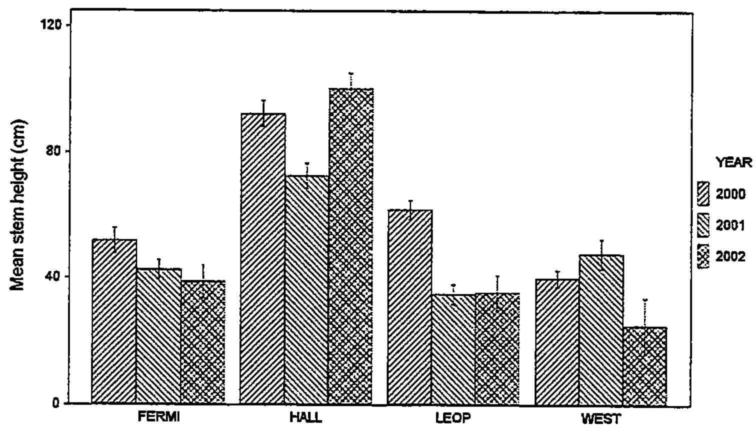

Fig. 56. Garlic mustard mean stem height (cm) in May at all sites, 2000 - 2002.

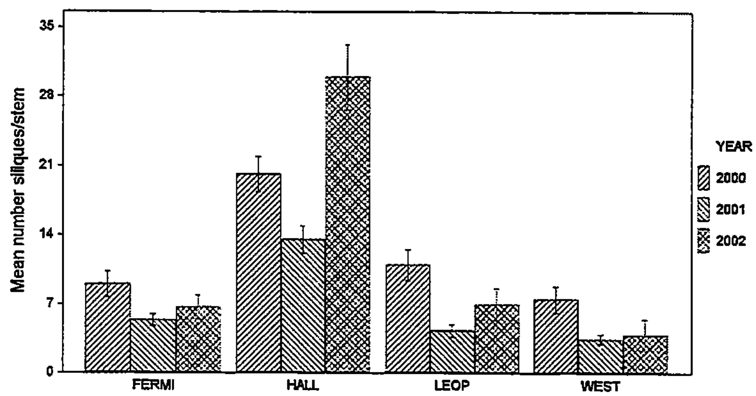

Fig. 57. Mean number of siliques/stem/0.5m^2 in June at all sites, 2000 - 2002.

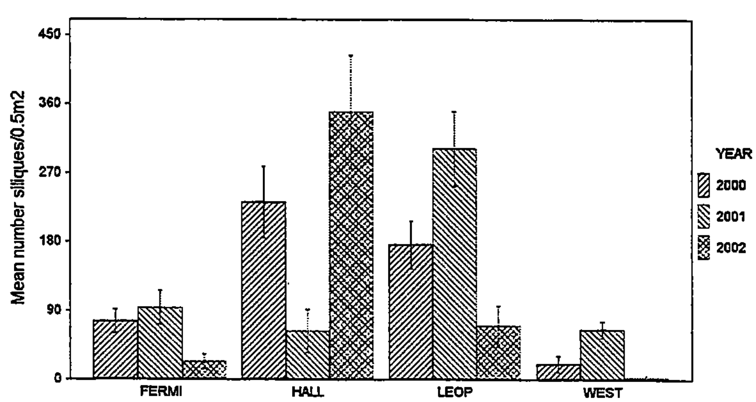

Fig. 58. Mean number siliques/0.5m^2 in June at all sites, 2000 - 2002.

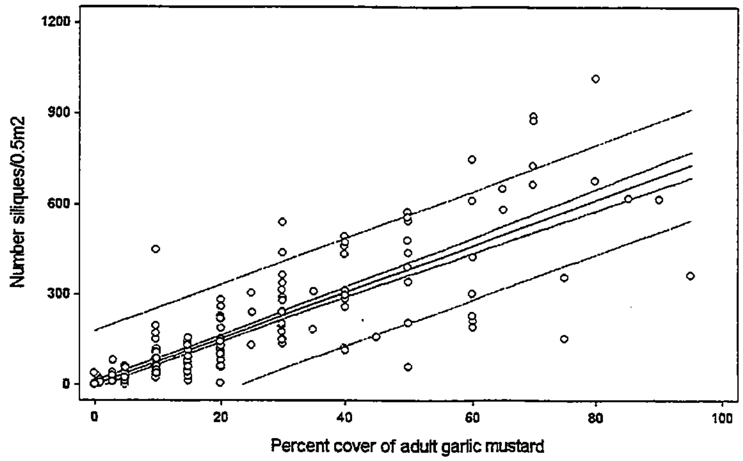

Fig. 59. Number siliques/0.5m^2 regressed against percent cover of adult garlic mustard in June, all sites and years combined.

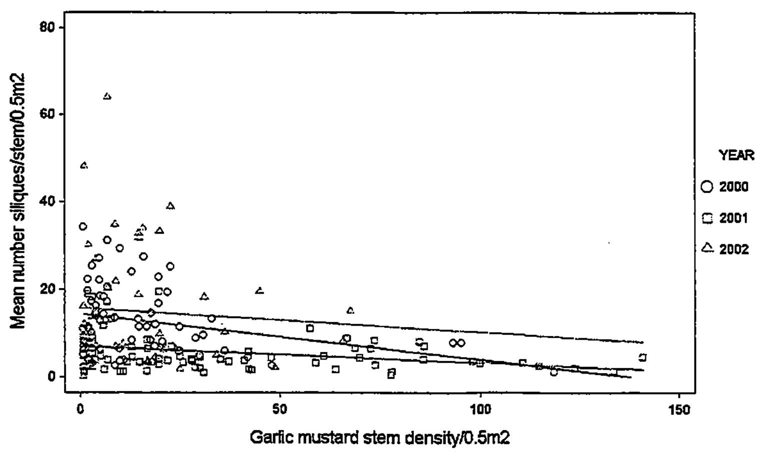

Fig. 60. Mean number siliques/stem regressed against adult stem density in June at all sites, 2001 – 2002.

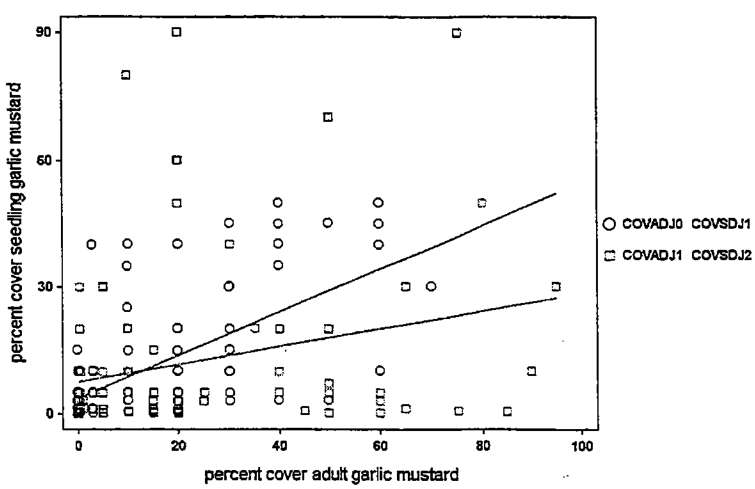

Fig. 61. Garlic mustard seedling cover in June as a function of adult cover in June of the previous year.

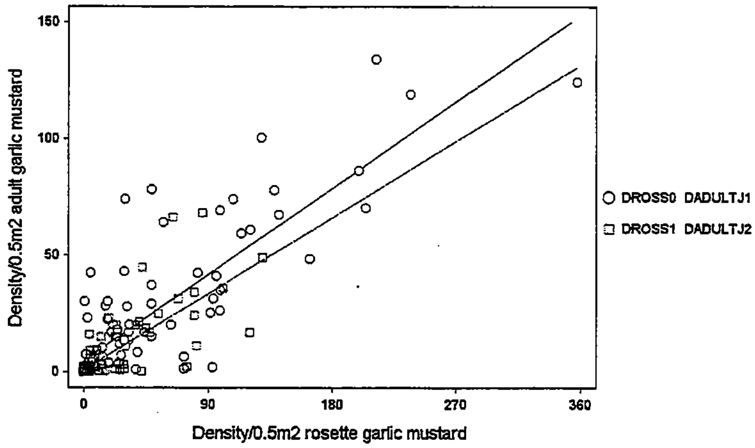

Fig. 62. Garlic mustard adult density/0.5m^2 in June as a function of rosette density/0.5m^2 the previous October.

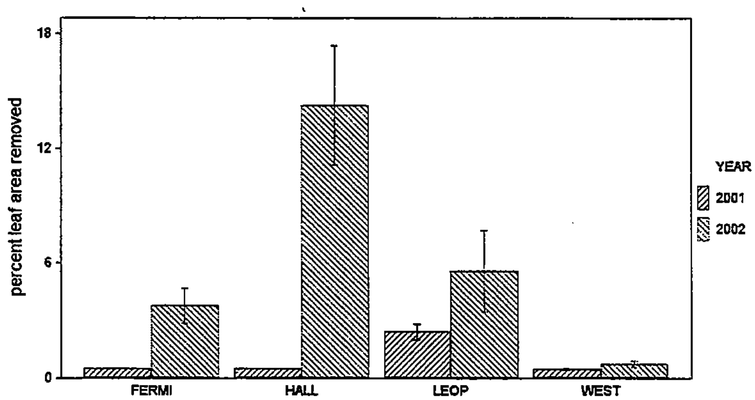

Fig. 63. Percent of garlic mustard leaf area removed by herbivory and disease in June at all sites, 2001 – 2002.

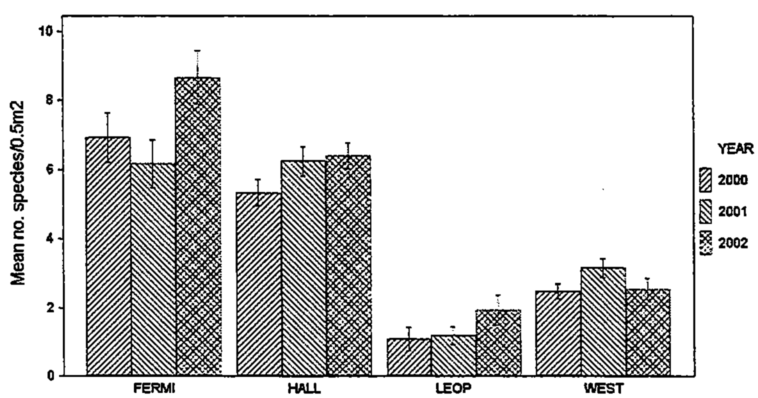

Fig. 64. Mean number species (excluding garlic mustard)/0.5m^2 in June at all sites, 2000 - 2002.

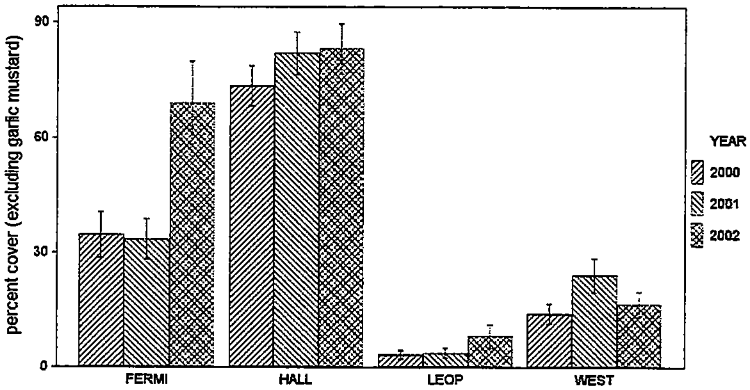

Fig. 65. Percent cover of groundlayer vegetation (excluding garlic mustard) in June at all sites, 2000 - 2002.

DISCUSSION

We tested and evaluated use and validity of multiple measures to develop a standardized monitoring protocol that could be used to gain insight into, and quantitative data about, the impact of proposed biocontrol on garlic mustard, and would allow wide-spread adoption and implementation across the range of garlic mustard. Numerous parameters (leaf size, biomass, etc.) predict fecundity of individual plants, but are too elaborate or time consuming to use as monitoring measurements. The initial monitoring protocol was based on garlic mustard's biology and the biology of the four weevils considered as biocontrol agents. Testing and improvement of the protocol was based on pre-release conditions.

The initial measures selected proved valid for the most part. Garlic mustard presence was consistently high, indicating that permanent quadrats functioned as predicted and are suitable for long-term monitoring. The 0.5 m^2 quadrat size proved more effective than the smaller 0.25 m^2 quadrat in reducing within-site and between-year variation in garlic mustard, and for capturing presence of garlic mustard. Given the variable presence of *Alliaria* due to biennial nature (high density one year can be followed by low density the next) and natural mortality (summer drought and overwinter mortality), the 0.5 m^2 quadrat is maintained in the monitoring protocol. A larger quadrat (1 m^2) might reduce (but not eliminate) the high variation, but time required to collect data would increase. The recommended number of quadrats was set at 20 to balance time-requirements with the need to obtain sufficient data, given the inherent variability of garlic mustard.

Cover of adult garlic mustard was a good predictor of silique production/0.5 m^2, and height of garlic mustard was strongly correlated with number siliques/stem. Thus, we retained both measures. We also recommend continuing to count siliques on all stems as an indication of biocontrol impact, i.e., reduction in seed production. We can't predict exactly how biocontrol will impact garlic mustard in North America, nor how attack will affect the relationship between silique production and both cover and stem height.

While stem density varied from year to year within sites, mean stem height was relatively similar at each site over the three-year study period. This suggests that stem height may be a relatively stable measure, regardless of garlic mustard abundance in any given year. Since stem height is sensitive to heavy attack of the proposed biocontrol insects, declining 10-15 cm in tests conducted in Switzerland, it is possible that a consistent and significant reduction in stem height following introduction of the weevils could be attributed to, and used as an indication of, weevil establishment and impact.

Community measures (percent cover and species richness) showed that the invaded community remained stable over the three-year study. Species richness is easier to record than species cover and is consistent among practitioners provided they are familiar with species identification, but is very resistant to change. Species cover is more time-consuming to record, and can vary depending on the investigator, but provides considerably more information than just presence, and is more sensitive to change than species richness. Both measures are strongly recommended for inclusion in monitoring, when practitioners are able to identify the resident plant species.

From European fieldwork and experiments, we know that garlic mustard responds to herbivore attack by decreasing in height, sometimes producing more but smaller diameter stems, sometimes producing more inflorescences and more siliques, and producing fewer seeds/plant. The actual impact of biocontrol on garlic mustard in North America needs to be evaluated after release, and cannot be predicted accurately.

Every measure of garlic mustard abundance showed extreme fluctuation from year to year, reflecting the natural variation in this biennial species. As a result, identifying long-term trends, and separating the impact of biocontrol from natural fluctuations, will require more than the three years reported here. Therefore, we strongly recommend that pre-release monitoring be initiated as early as possible and be conducted annually. Initiating monitoring one year prior to release of biocontrol will not provide adequate baseline data to detect a biocontrol-related impact.

There is some indication that garlic mustard populations are highest early in the invasion, and then level off. Similarly, rosette size, stem density and silique density also appear to be higher in the early stages of invasion than later on. Only monitoring many sites over many years will allow us to avoid erroneously concluding an impact of biocontrol agents when in fact the decline in population was caused by other (yet to be identified) factors. In addition, incorporating data from multiple locations will facilitate regional and national assessments of biocontrol effectiveness, and offer insights into factors beyond what can be determined from just a few sites.

Testing was conducted under real-world conditions, and included unexpected occurrences: Two quadrats were lost at Hall Woods due to vandalism, and one was lost to tree fall. At Fermilab, a management fire burned through the study site in fall 2001, and burned many of the rosettes. These types of events are not atypical, and the monitoring protocol appears to be robust enough to accommodate unexpected events.

The final Monitoring Protocol (Appendix B) gives detailed information on site selection, quadrat construction and layout, and data collection. Data sheets are also included, along with a 'check list' for each sheet, an equipment list, and additional information. Identifying the cause of damage is important to 1) verify presence of and feeding intensity by, the biocontrol weevils, and 2) accurately assign the cause of any decline in garlic mustard to the correct factor(s).

Using this protocol, practitioners can begin monitoring garlic mustard infestations well before release of biocontrol weevils. The resulting baseline data will permit a BACI study design (Before and After introduction, in Control and Impact areas) and help to separate the impact of biocontrol insects from other factors. Once insects are released, practitioners will be able to determine if the insects have established; document 'success' or lack of success at a site; and help track changes in the natural community following biocontrol.

Since June 2002 a number of investigators have implemented this monitoring protocol. Training workshops will be offered in June 2003 in the Chicago region to introduce the protocol to natural area managers in the Midwest. The monitoring protocol will be available to other practitioners on the http://www.invasiveplants.net website created and maintained by the Ecology and Management of Invasive Plants Program at Cornell University.

After biocontrol insects have been approved for release, additional monitoring methods will be field tested for usefulness in detecting presence and abundance of biocontrol weevils, such as timed counts for adults, and number of feeding marks, and incorporated into an updated version of the monitoring protocol.

Technology transfer

Even before the start of this SERDP project, we had developed a list of collaborators across North America. The first drafts of a host specificity list were circulated widely through North America to gain feedback on our initial test plant selection. A test plant list of approximately 50 species taxonomically related to *A. petiolata*, occurring in the same habitat, with chemical similarities, and important agricultural plants was finalized with the received input. We have maintained these contacts throughout the duration of this grant and disseminated information widely through various means. This network of contacts has allowed us to accomplish a nationwide quantitative survey for potential enemies of garlic mustard in North America through samples received from 49 different locations. We gave presentations at many different venues in North America and also in Europe. Among the target audiences were scientific societies, natural area managers, as well as participants in many workshops or garden clubs. In addition we published a summary of our efforts (predating the SERDP grant) in a paper published in the Natural Areas Journal in 2001. I co-edited a Technology Transfer book published by the Forest Health Technology Enterprise Team on Biological Control of Invasive Weeds in the Eastern United States and we co-wrote the chapter on garlic mustard (a copy of this book is enclosed with this report). We believe our audience at military installation as well as the many natural area managers across North America is well aware of the ongoing efforts. The webpage maintained by my program at Cornell University (www.invasiveplants.net), is another vehicle to reach those interested in biocontrol, and the garlic mustard efforts in particular.

These efforts have contributed greatly to the seamless continuation of this project with funding contributed through the US Forest Service. A meeting was held in Minneapolis in spring 2002 to discuss the needs of the garlic mustard biocontrol program with wide participation from agencies and institutions in the Northeast and Midwest. Additional host specificity tests needed (see host specificity section and below) are under way in Europe. A new quarantine facility opened in Minneapolis, MN in the last year will be utilized for tests using North American plants difficult to grow in Europe. Two scientists from Minnesota are planning a visit to CABI in March 2003 to learn techniques and familiarize themselves with the insects. Tests are scheduled to begin in the fall 2003 with a focus on *C. scrobicollis*.

The development of a standardized monitoring protocol has been received with enthusiasm by collaborators. In June 2003 we introduced 30 natural area managers to the procedures during a two-day workshop in Ithaca, NY. We have also advertised the upcoming availability of this protocol at the many presentations we gave across the country. This has resulted in recent requests to send draft protocols to those interested in beginning monitoring early. Efforts are under way in MN, WI, IL, MI, IN, NY and by agencies such as the US Forest Service to establish long-term sites. Ideally, monitoring should be implemented a few years before releases are actually carried out and this concept has been embraced by a number of those interested in control of garlic mustard. We believe that our efforts in development and implementing more sophisticated long-term monitoring in weed biological control programs protocol, which began with a standardized monitoring protocol for purple loosestrife nearly 10 years ago, are surprisingly well adopted. This will be to the benefit of evaluations of biocontrol programs and contribute to the improvement and safety of the discipline. We will make the protocol be available in pdf-format at the website of the Ecology and Management of Invasive Plants Program (www.invasiveplants.net) at Cornell University and intend to publish the most recent version in a biocontrol journal (see below).

We will continue to evaluate the vast amounts of data accumulated over the 3 years of this project. The following provides a list of the anticipated publications to result from this work. Authorship will be shared (where appropriate) among all or part of the investigators.

1. Niche overlap of *Ceutorhynchus alliariae* and *C. roberti*, two specific shoot-miners co-occurring on *Alliaria petiolata*. (to be submitted to: Oikos, Oecologia, or Ecology)
2. *Ceutorhynchus alliariae* and *Ceutorhynchus roberti*: Coexistence and competitive interactions between two potential biological control agents of garlic mustard, *Alliaria petiolata*. (Brassicaceae) (to be submitted to: Oikos, Oecologia, or Ecology)
3. Impact of two shoot-mining weevils (*Ceutorhynchus alliariae* and *C. roberti*) on the invasive plant *Alliaria petiolata*. (to be submitted to: Journal of Applied Ecology, Environmental Entomology, or Weed Science)
4. Impact of the below-ground herbivore *Ceutorhynchus scrobicollis* on *Alliaria petiolata*. (to be submitted to: Journal of Applied Ecology, Environmental Entomology, Weed Science)
5. Combined effects of above- and below-ground herbivores on *Alliaria petiolata*. (to be submitted to: Journal of Applied Ecology, Environmental Entomology, or Weed Science)
6. Biology and host specificity of *Ceutorhynchus scrobicollis*, a potential biological control agent of *Alliaria petiolata* in North America (to be submitted to: Biological Control)
7. Host range of *Ceutorhynchus alliariae* and *Ceutorhynchus roberti,* two potential biological control agents of *Alliaria petiolata* in North America. (to be submitted to: Biological Control)
8. Biology and host specificity of *Ceutorhynchus constrictus*, a potential biological control agent of *Alliaria petiolata* in North America (to be submitted to: Biological Control)
9. Distribution and abundance of natural enemies of garlic mustard (*Alliaria petiolata*) in North America (to be submitted to Biodiversity and Distributions)
10. High seed predation by native herbivores is unable to prevent spread of an invasive biennial mustard (*Alliaria petiolata*) in North America (to be submitted to: Oikos).
11. Development of a standardized monitoring protocol to assess success of biological control for garlic mustard (*Alliaria petiolata*). (to be submitted to: Biological Control)

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Appendix A

Proposed test plant list for host specificity testing of potential biocontrol agents on garlic mustard (*Alliaria petiolata*) and reasons for inclusion. (• denotes a native North American species)

Species	Taxono-mically related	Occurs in the same habitat	Similar plant chemistry	Important crop plant
Brassicaceae				
Armoracia rusticana (*A. lapathifolia*), (horse radish)	X		X	X
Aubrieta columnae	X			
Barbarea vulgaris (wintercress)	X		X	
Brassica napus napus (oilseed rape)	X		X	X
Brassica nigra (black mustard)	X		X	X
Brassica oleracea gemmifera (brussels sprout)	X			X
Brassica oleracea italica (broccoli)	X			X
Brassica rapa rapa (turnip)	X		X	X
Capsella bursa-pastoris (shepards purse)	X		X	
Hesperis matronalis (dame's rocket)	X	X		
Nasturtium vulgaris (water cress)	X			
Raphanus sativus (radish)	X		X	X
Reseda lutea	X		X	
Rorippa sylvestris (creeping yellow cress)	X			
Sinapis alba alba (white mustard)	X		X	X
• *Arabis canadensis* (sickle pod)	X	X		
• *Cardamine bulbosa* (bulbous cress)	X	X		
• *Cardamine pensylvanica* (pennsylvania bittercress)	X	X		
• *Dentaria laciniata* (toothwort)	X	X		
• *Draba reptans* (common whitlow grass)	X			
• *Lepidium virginicum* (common peppergrass)	X			
Poaceae				
• *Elymus hystrix* (bottlebrush grass)		X		
• *Zea mays* (corn)				X
Triticum aestivum (wheat)				X
Fabaceae				
Glycine max (soybean)				X
• *Amphicarpaea bracteata* (hog peanut)		X		
Cyperaceae				
• *Carex laxiflora* (*C. blanda*), (common wood sedge)		X		
Liliaceae				
• *Allium canadense* (wild garlic)		X		
• *Allium tricoccum* (wild leek)		X		
• *Trillium grandiflorum* (large flowered trillium)		X		
• *Smilacina racemosa* (false solomons seal)		X		
• *Erythronium americanum* (trout lily)		X		
Araceae				
• *Ariseama triphyllum* (jack-in-the-pulpit)		X		
Aristolochiaceae				
• *Asarum canadense* (wild ginger)		X		
Portulacaceae				
• *Claytonia virginica* (spring beauty)		X		
Apiaceae				
• *Osmorhiza claytonii* (sweet cicely)		X		
Rubiaceae				
• *Galium aparine* (cleavers bedstraw)		X		

Species (continued)	Taxono-mically related	Occurs in the same habitat	Similar plant chemistry	Important crop plant
Papaveraceae				
• *Sanguinaria canadensis* (bloodroot)		X		
Fumariaceae				
• *Dicentra cucullaria* (dutchmans breeches)		X		
Geraniaceae				
• *Geranium maculatum* (wild geranium)		X		
Berberidaceae				
• *Podophyllum peltatum* (mayapple)		X		
Hydrophyllaceae				
• *Hydrophyllum virginicum* (virginia waterleaf)		X		
Vitaceae				
• *Parthenocissus quinquefolia* (virginia creeper)		X		
Asteraceae				
• *Solidago flexicaulis* (zig-zag goldenrod)		X		
Ranunculaceae				
• *Isopyrum biternatum* (false rue anemone)		X		
• *Ranunculus septentrionalis* (swamp buttercup)		X		
Violaceae				
• *Viola sororia* (blue violet)		X		
Polemoniaceae				
• *Phlox divaricata* (woodland phlox)		X		
Polygonaceae				
• *Polygonum virginianum* (woodland knotweed)		X		

Literature consulted:

Fernald, M.L. 1970. Gray's manual of botany, 8th edition. Van Nostrand, NY.

Gleason, H. A., and A. Cronquist. 1991. Manual of vascular plants of Northeastern United States and adjacent Canada, 2nd edition. The New York Botanical Garden, NY.

Hegi, G. 1986. Illustrierte Flora von Mitteleuropa, Band IV. Paul Parey, Berlin.

Swink, F., and G. Wilhelm. 1994. Plants of the Chicago region. 4th edition, Indiana Academy of Science, Lisle, IL.

Appendix B

Garlic Mustard Monitoring Protocol

Final Draft/ 20 October 2002

Introduction:

Garlic mustard (*Alliaria petiolata*) is a biennial European herb that invades forested communities in North America, especially in the central and eastern part of the US and adjacent Canada. A biological control program targeting garlic mustard was initiated in 1997, and releases of the first insects are anticipated to begin in 2004. The following guidelines are intended to help monitor the abundance of both garlic mustard and the biocontrol insects, and assess the long-term impact of biological control. For maximum information, monitoring should ideally be initiated one or more years before biocontrol organisms are released: the resultant 'pre-release' data will provide a baseline to monitor 'post-release' changes.

We would appreciate your feedback on this monitoring protocol.

Background:

This monitoring protocol is based on garlic mustard's biology, and the biology of the four weevils considered as biocontrol agents (see sidebar I). For best results, monitoring should be conducted twice a year; in June to assess plant density and seed production, and in October to assess rosette abundance and external evidence of insect feeding. This monitoring protocol is designed to detect spread of the biocontrol weevils and their impact on garlic mustard. The protocol can also be used to detect change in herbaceous vegetation relative to change in garlic mustard.

Garlic mustard is an obligate biennial and can only spread by seeds; therefore the goal of biocontrol is population reduction, achieved by reducing total seed production. Garlic mustard seeds germinate in early spring, and form a basal rosette by June. Plants remain as rosettes through the winter, and produce flower stalks the following spring, usually blooming in April - May, depending on the location and temperature regime. Seeds are produced in siliques (linear pods) 4-8 weeks later, usually in June-July. Garlic mustard seeds live 3-5 years in the seedbank.

The four weevils (*Ceutorhynchus* sp) are difficult to observe directly; adults are small, and larvae feed inside the plants (in seeds, stems, leaves, and root crowns). However, all four weevils produce a characteristic 'window pane' feeding pattern that can be easily observed on the leaves (Sidebar I). Under heavy attack by one or more of the weevil species, garlic mustard plants become shorter and less robust, often have tip dieback, and produce fewer flowers and siliques.

Site Selection:

Select a monitoring site that will be protected from all other uses and expected natural disturbances (such as flooding), for a minimum of ten years. It is imperative that the monitoring site be protected from all management that could damage the insects or the garlic mustard plants, in particular burning, herbicide application, and pulling of plants. We do not know how these weevils will respond to fire or flooding, and in the initial establishment phase a fire (which may burn the insects), flooding (which may drown the insects), or removal of garlic mustard plants (with the insect larvae hidden inside) could eradicate the population. The study site should be sufficiently distant from a trail to limit vandalism.

The study site should be a forested community at least 2 ha in size, with an established garlic mustard population. Garlic mustard does not need to form a continuous carpet, but should be present throughout the study area every year, as rosettes and/or adult plants. To determine response of the associated groundlayer vegetation to the anticipated reduction in garlic mustard, it would be beneficial to locate the study site in an area with native vegetation. Avoid establishing plots in a site where garlic mustard has been present for <3 years, as the population should be large enough with a well-established seed bank to maintain a reliable food source for these weevils.

Quadrat Setup:

We recommend a total of 20 permanent $0.5m^2$ (0.5m x 1.0m) quadrats, spaced ≥10 meters apart. This allows statistical analysis, and provides sufficient locations to ensure that garlic mustard is present as adult or seedling in most quadrats each year (in general, once garlic mustard is present, it will continue to be present almost every successive year in that location, although densities may vary significantly).

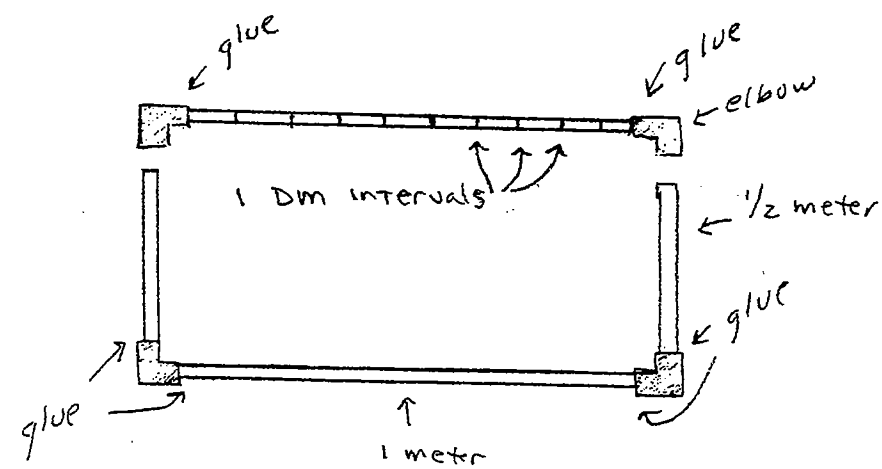
Quadrats can be located in several ways: along two parallel transects, in 4 rows of 5 quadrats, or completely randomly. Relocating the quadrats is easier using parallel transects, and this method will be outlined here. Randomly establish two parallel transects, at least 100m long and ≥10 meters apart. Locate quadrats at fixed intervals ≥10 meters apart along each transect, making sure that garlic mustard occurs in every quadrat. Shift the quadrat location as needed to have garlic mustard cover at least 25% of each quadrat. In sites where both age classes (adults and rosettes) are present, have both age classes represented in the 20 quadrats.

We recommend using a 'u' shaped quadrat frame, with three sides fixed and the fourth removable (see sidebar 2 for construction). To establish the permanent quadrats, first locate the position of each quadrat, then place the quadrat frame on the ground, and mark the four corners by hammering a 12-18" long and 1/2" diameter plastic or aluminum conduit in each corner. This will allow exact placement of the quadrat in future years. Write the quadrat number on each conduit with a permanent marker or other means. In areas with high public use and potential vandalism, conduits should be short and difficult to see. Obvious markings can attract vandalism and 'helpful protectors' who remove the conduits. Avoid trampling vegetation in and near the quadrat. If tall vegetation is present when quadrats are being established, slide the open-ended u-shaped frame along the ground to avoid disturbing the vegetation. Then, attach the fourth side to the frame.

Sidebar 1: Four weevil species are under study for introduction as biocontrol agents: one seed-feeder (*Ceutorhynchus constrictus*), two stem-feeders (*C. alliariae* and *C. roberti*), and one root crown feeder (*C. scrobicollis*). Larvae induce most of the damage, but because they feed inside the plant they are not usually observed. Adults feed externally and create a characteristic 'windowpane' feeding pattern, especially in autumn (*C. scrobicollis*). In addition, adults feed on stems and petioles, leaving a 'scraping' mark. All adults are small (2mm) and black.

Feeding pattern:

Sidebar 2: We recommend an open-ended frame with the fourth side removable. Construct the quadrat frame from a 10' length of 1/2" diameter pvc or cpvc pipe, 4 right-angle elbows of the same diameter, and pvc or cpvc glue. The inside dimensions of the frame should measure 1m by 0.5m. After cutting the conduit to the correct lengths, glue two elbows to each 1m long piece (make sure the elbows are perfectly aligned to each other). Set one piece aside (This will be the fourth side of the frame). Glue the elbows of the other 1m long piece to two 0.5m long pieces to form the open 'u' shaped frame. Using a permanent marker, mark 1 dm intervals on each side to assist with estimating percent cover. In the field, slide the open frame into position, and then attach the fourth side to it.



Data Collection:

Four data forms are provided: site location form, summer monitoring form (two pages), and autumn monitoring form.

Form I: Site location, background information

Site Location:

Enter name of the site (for example: Fillmore Glen State Park, north unit: be as specific as possible); and the location (town, county, state, etc.). If Global Positioning System (GPS) coordinates are available, enter this information in the spaces provided.

Contact Person and Legal Landowner:

Provide the name, address and telephone number of a contact person. This person can be the releaser or a local contact. If the contact person is not the legal landowner, please provide this information in addition.

Site Characteristics:

Check one of the options or provide specifics if none of the options are applicable.

Road Map:

Photocopy a road map (preferably a county road map) to the site from a Road Atlas and paste it into the space provided. Mark the location of the site. An arrow should indicate North on map. If a written description of directions is needed, attach the description to this page. Be specific: assume the reader has never been to the locale. Attach additional pages if needed

Site and Vegetation Map:

Provide a map of the area with access roads, approximation of garlic mustard infestation outlined, other vegetation types, trails, creek etc. Paste map into space provided. If insects have been released, indicate with Arabic numerals (corresponding to numbers under Insect Releases) points of single or multiple control agent releases. An arrow should indicate North on the map.

Photographs of changes in vegetation over time are a powerful tool for presentations or to re-enforce quantitative data. One or several permanent photo-points should be marked in the area of insect release(s) using flagging tape or stakes driven into the ground. The position of these photo-points should be indicated on the vegetation map. The direction in which the picture was taken should also be indicated with an arrow. Take pictures once a year at the same time of the year. The showy flowers of garlic mustard suggest taking pictures at the peak of the flowering period. Make sure to record which photos were taken from which location and when.

Insect Release History:

Document date, control agent species, life stage (adults, eggs or larvae), the number of individuals released, and how individuals were released, as well as time of day and weather conditions. Use additional sheets if necessary. Code each release with an Arabic numeral and insert number at the release point on the vegetation map (see above).

FORM 1: SITE LOCATION:

Site Name: ______________________________ Date: ________
Town: ________________ County: ______________ State: ________
Longitude: __________ Latitude: __________ GPS Derived? Y N
Elevation: _____ Range: ______ Twnship: __________ Sect: ________ QtrSect: ________

CONTACT PERSON:
Name: ______________________
Address: ______________________
City: ______________________
State: __________ Zip: __________
Phone: ______________________

LEGAL LANDOWNER:
Name: ______________________
Address: ______________________
City: ______________________
State: __________ Zip: __________
Phone: ______________________

SITE CHARACTERISTICS:
Habitat Type: __River __Wetland __Lake __Meadow __Irrigation Ditch __Other

Road Map to Site

N

Site and Vegetation Map

N

INSECT RELEASE HISTORY:

Date (mm/dd/yy)	Species	Number and Stage (egg/larvae/adult)	Position of Release on Map (1,2,3,4...)

Form 2a and 2b: Summer Monitoring

Materials needed: I meter stick; 0.5m² quadrat frame; data sheets (Form 2a and several copies of Form 2b), and pencils and a clipboard.

Summer data should be recorded when garlic mustard has completed flowering and has fully formed green siliques, but before the siliques turn brown and start to disperse seed. In northern locales this is usually in mid to late June, while in southern locales this may be as early as mid May.

Use new data sheets each time. Before collecting data, please record in spaces provided: site name, date, and the names of the observers, as well as general weather pattern (sunny, overcast, rainy, humid), temperature, and time of day of observations. To assess the growth and seed production of garlic mustard, and growth of other groundlayer species, a series of estimates are used. All estimates reflect the growth within each quadrat and NOT of the site as a whole, or plants near but not in the quadrat.

Begin with quadrat I and fill out both Form 2a, and then Form 2b (if adult garlic mustard are present), then move to the next quadrat. Summer monitoring is easier with two people, one to make the observations and the other to record data.

<u>Form 2a: Garlic Mustard Biocontrol Monitoring (Summer)</u>

I) First, slide the frame into position. Standing over the frame, and looking straight down, estimate how much of the quadrat is covered by garlic mustard and, independently, how much is covered by all other vegetation (Use cover estimates in Chart A, or a finer scale (for example. Present; <I% cover; 2-5% cover, and in I0% increments thereafter i.e.; >5-I5%, >I5-25%, etc). If both garlic mustard and other vegetation are abundant, these estimates may total >I00%, due to layering. Next, focus only on garlic mustard. If adult garlic mustard plants are uncommon or small, or if only seedlings are present, you may need to carefully move vegetation to determine how much garlic mustard is actually present in each age class. Estimate the actual percent cover (using the cover classes in Chart A) of all garlic mustard; of only adult garlic mustard; and of only seedling garlic mustard. Often, adult garlic mustard will overtop seedling garlic mustard, and their combined cover will therefore exceed the 'all garlic mustard' cover. That is okey, as we are interested in monitoring how much of each size class is present.

2) Next, scan the garlic mustard for any damage to the leaves, shoots, or siliques. After insect release, look especially for the 'window pane' feeding pattern of the biocontrol weevils. Estimate the percent leaf area of garlic mustard removed by insect feeding integrated over the entire quadrat, using Chart A. Initially, this will be very low or non-existent. After weevil populations build up you may find as much as 50% of the leaves are damaged. Next, indicate what type of damage is visible, such as leaf miners, deer browse, disease, etc., using a 'check' or '+' in the appropriate box. This may be omitted if feeding damage is very low (<I%) and not clearly discernible. Make a note if some other type of damage is present, and include a sketch or photograph of the damage.

Estimating the amount of leaf area removed by insect feeding will initially be difficult because you need to scan through the vegetation, and leaves and plants will show different amounts of feeding damage, but you will get better over time. Experienced observers should introduce new personnel to the methods and to their assessments to increase the accuracy of reported results. We expect to observe large differences over time, especially following high abundance of *Ceutorhynchus* larvae and adults. With the proposed methods, we will be able to assess these changes.

3) Count the number of seedlings. If seedling density is very high, count the number of seedlings in a section of the quadrat, and then use this density to estimate the total number of seedlings in the quadrat.

If time does not allow counting individuals or a subset of the population, use Chart B to estimate seedling density. Estimations are never as accurate or powerful as actual counts, so count actual seedling density whenever possible.

4) Looking below all vegetation, estimate the cover of soil, wood, leaves and rock using Chart A. This should total 100%. Often, sites with abundant garlic mustard have little leaf litter.

5) Measure litter depth to the closest cm in the center of each half-quadrat.

6) If you are interested in monitoring the associated groundlayer vegetation, record presence (and estimated percent cover) of all species rooted in the quadrat. Use cover estimates in chart A, or a finer scale (for example. Present; <1% cover; 2-5% cover, and in 10% increments thereafter i.e.; >5-15%, >15-25%, etc).

Other Observations:

Record any general observations or useful information about the site; windfall, flooding, deer herbivory, insects etc. Most of this information will be difficult to evaluate, so do not spend too much time on this.

Form 2b: Garlic Mustard Biocontrol Monitoring (Adult height and number siliques)

Use this form when adult garlic mustard are present in the quadrat. Write the quadrat number in the appropriate box at the top of the sheet. Then, beginning at one corner of the quadrat and working systematically across the quadrat, measure the height, and count the number of siliques, of each garlic mustard stem. Record this information in the appropriate boxes below the quadrat number. Record each stem that originates from the ground as a separate stem, even if you suspect that some stems may originate from a single root. When a stem branches >2cm above the ground, then the branch is counted as part of the single stem. Also, look carefully for short, frequently sterile stems. These small plants are usually overlooked, but it is important to record their presence. Record every stem, using several columns if necessary, and writing the quadrat number above each column. To be counted, a stem must originate within the quadrat; if it originates under the frame, then it is not recorded.

If you see overt damage or anything unusual on a stem, you can record this in the same box, by using an asterix, or a letter, or other symbol, and defining it in the box labeled "notes". For example, if you see leaf mining on a stem 30cm tall with 7 siliques, you could record this by writing "30-7 *" on the data sheet and writing in the notes box "* = leaf mining".

It is important to measure every stem in the quadrat, even if some quadrats have numerous plants. We anticipate that under heavy insect attack garlic mustard plants will decrease in density, height, and silique production, and will also change in plant architecture and produce more small side branches. Therefore it is very critical to have accurate baseline data to compare to 'post-release' data, and accurately assess the impact of the weevils on garlic mustard.

Please mail or fax a copy of the completed form to:

Dr. Bernd Blossey
Assistant Professor and Director
Biological Control of Non-Indigenous Plant Species Program
Department of Natural Resources
122E Fernow Hall, Cornell University
Ithaca, New York 14853 USA

phone: 607-255-5314
fax: 607-255-0349
homepage: http://www.invasiveplants.net

Form 3: : Garlic Mustard Biocontrol Monitoring (Autumn)

Materials needed: I meter stick; 0.5m^2 quadrat frame; data sheets (Form 3), and pencils.

These are similar measures to those collected in summer, except that flower stem density and height are not measured. Because only one size class (rosette) is present, the autumn monitoring takes less time than the spring monitoring, and can be conducted by one individual.

Monitoring should occur about the time deciduous trees lose their leaves. Indicate in the 'notes' box whether trees have lost some, all, or none of their leaves (this helps with interpretation of leaf litter depth, and of garlic mustard percent cover, as small rosettes are often covered by new leaves and will be missed in sampling).

I) First, if insects have been released, approach the quadrat slowly and observe for weevils. Typically, only the rosette-feeder *C. scrobicollis* will be active at this time. You may see these small (2 mm) black insects near the center of a rosette.

2) Next, slide the frame into position. Standing over the frame, and looking straight down, estimate how much of the quadrat is covered by garlic mustard and, independently, how much is covered by all other vegetation (Use cover estimates in Chart A, or a finer scale (for example. Present; <I% cover; 2-5% cover, and in I0% increments thereafter i.e.; >5-15%, >I5-25%, etc)). If rosettes are uncommon or small, or tall vegetation is present, you may need to carefully move vegetation to determine how much garlic mustard is actually present. If both garlic mustard and other vegetation are abundant, these estimates may total >I00%, due to layering. That is okey, as we are interested in monitoring how much of each is present.

3) Next, scan the garlic mustard for any damage to the leaves, shoots, or siliques. After insect release, look especially for the 'window pane' feeding pattern of the biocontrol weevils. Autumn is when this feeding pattern is most distinct if the rootcrown feeder *C. scrobicollis* is present Estimate the percent leaf area of garlic mustard removed by insect feeding integrated over the entire quadrat, using Chart A. Initially, this will be very low or non-existent. After weevil populations build up you may find as much as 50% of the leaves are damaged. Next, indicate what type of damage is visible, such as slugs (round holes >I cm diameter), deer browse, disease, leaf miners, etc. using a 'check' or '+' in the appropriate box. This may be omitted if feeding damage is very low (<I%) and not clearly discernible. Make a note if some other type of damage is present, and include a sketch or photograph of the damage.

Estimating the amount of leaf area removed by insect feeding will initially be difficult because you need to scan through the vegetation, and leaves and plants will show different amounts of feeding damage, but you will get better over time. Experienced observers should introduce new personnel to the methods and to their assessments to increase the accuracy of reported results. We expect to observe large differences over time, especially following high abundance of *Ceutorhynchus* larvae and adults. With the proposed methods, we will be able to assess these changes.

4) Count the number of rosettes. If rosette density is very high, count the number of rosettes in a section of the quadrat, and then use this density to estimate the total number of rosettes in the quadrat. If time does not allow counting individuals or a subset of the population, use Chart B to estimate rosette density. Estimations are never as accurate or powerful as actual counts, so count actual rosette density whenever possible.

5) Looking below all vegetation, estimate the cover of soil, wood, leaves and rock using Chart A. This should total I00%. Often, sites with abundant garlic mustard have little leaf litter.

6) Measure litter depth to the closest cm in the center of each half-quadrat.

7) If you are interested in monitoring the associated groundlayer vegetation, record presence (and estimated percent cover) of all species rooted in the quadrat. Use cover estimates in chart A, or a finer scale (for example. Present; <1% cover; 2-5% cover, and in 10% increments thereafter i.e.; >5-15%, >15-25%, etc).

Other Observations:
Record any general observations or useful information about the site; windfall, flooding, deer herbivory, insects etc. Most of this information will be difficult to evaluate, so do not spend too much time on this.

Please mail or fax a copy of the completed form to:

Dr. Bernd Blossey
Assistant Professor and Director
Biological Control of Non-Indigenous Plant Species Program
Department of Natural Resources
122E Fernow Hall, Cornell University
Ithaca, New York 14853
USA

phone: 607-255-5314
fax : 607-255-0349
homepage: http://www.invasiveplants.net

Summer Monitoring Quick Reference (Forms 2A and 2B)

1. Materials: 1 meter stick; m^2 quadrat frame; data sheets (Form 2A and several copies of form 2B); pencils.

2. Walk to quadrat 1. Slide quadrat frame into location. Fill out Form 2A first, then Form 2B.

Form 2A:

3. Write Site name, date, and names of investrigators, state, and GPS coordinates if known.
4. Estimate Vegetatioin Cover: Use Chart A.
   a. Estimate total vegetation cover (maximum 100%). Write "0" if no vegetation present.
   b. Estimate Total garlic Mustard Cover. Write "0" if no garlic mustard present.
   c. Estimate cover of adult garlic mustard. Write "0" if no adult garlic mustard present.
   d. Estimate cover of seedling garlic mustard. Write "0" if no seedling garlic mustard present.
5. Look for evidence of leaf attack.
   a. Estimate percent of garlic mustard leaf area removed by insect feeding, estimated over the entire quadrat (use Chart A).
   b. Indicate type of damage visible and/or insects present in quadrat: check or write "+" for each type present.
6. Count the number of garlic mustard seedlings present in the quadrat. If too many to count, estimate density using Chart B.
7. Measure litter depth to the nearest 0.5 cm in the center of each half-quadrat.
8. Looking below all vegetation, estimate perecent cover of bare soil, leaf litter, down wood, and rock. Use Chart A or visually estimate so all 4 categories add up to 100%.
9. Optional Record presence (and estimated percent cover, if desired) of all plant species rooted in the quadrat. Use Chart A or other scale.

Form 2B:

10. If adult garlic mustard are present in the quadrat, fill out Form 2B:
    a. Write Site name, date, and names of investrigators, state, and GPS coordinates if known.
    b. Write quadrat number at top of the colomun. Start at one end of the quadrat and for each adult garlic mustard in the quadrat, record the:
       i. Height (in cm) of stem, measured to the top of the growing point.
       ii. Number of siliques (seedpods). Count only siliques that have at least one seed; do not count very small or empty siliques.
11. After completing Forms 2A and 2B for quadrat 1, proceed to quadrat 2, and repeat the process (steps 4-10, above). Continue until all quadrats have been located and recorded.

FORM 2a:
GARLIC MUSTARD BIOCONTROL MONITORING (Summer)

SITE ______________________ STATE__________

DATE______________________ GPS___________

Investigators______________________

please send a copy to: Bernd Blossey, Fernow Hall, Cornell University, Ithaca, NY 14853

Chart A: Percent cover & Damage class

A	<1%
B	1-5%
C	6-25%
D	26-50%
E	51-75%
F	76-95%
G	>95%

Chart B: Estimated Density

I	1-10
II	11-25
III	26-100
IV	100-500
V	>500

Notes:

	Q1	Q2	Q3	Q4	Q5	Q6	Q7	Q8	Q9	Q10	Q11	Q12	Q13	Q14	Q15	Q16	Q17	Q18	Q19	Q20
PERCENT COVER : (Use Chart A)																				
All Garlic Mustard																				
Adult GM																				
Seedling GM																				
All other vegetation																				
Leaf Attack (%removed) (Use Chart A)																				
Check if present: Leaf miner																				
Windowpane																				
Edge feeding																				
Holes																				
Spittle bug																				
Scale																				
Browse																				
Disease																				
Other																				
SEEDLING DENSITY: (use Chart B if too numerous to count)																				
PERCENT COVER OF: (Use Chart A or actual estimates that total 100%)																				
Soil																				
Wood																				
Leaf																				
Rock																				
LITTER DEPTH (cm)																				
SPECIES PRESENCE, % COVER																				

FORM 2B:

GARLIC MUSTARD BIOCONTROL MONITORING (Adult height and # siliques)

In each 0.5m quadrat, record:
Height (cm) and number siliques
of every adult garlic mustard
ex: 34-16 = 34 cm tall, 16 siliques

Notes:

SITE ____________________________

DATE____________________________

Investigators____________________

STATE____________

GPS_____________

please send a copy to: Dr. Bernd Blossey, Fernow Hall,
Cornell University, Ithaca, NY 14853

or fax to: 607-255-0349

QUADRAT #

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Autumn Monitoring Quick Reference (Form 3)

1. Materials: 1 meter stick; m^2 quadrat frame; data sheet (Form 3); pencils; stop watch
2. Write Site name, date, and names of investigators, state, and GPS coordinates if known at the top of Form 3.
3. Walk to quadrat 1. If insects have been released
    a. Approach the quadrat slowly and observe for weevils.. Slide quadrat frame into location.
    b. Indicate if weevils observed ('+" = present, "-" = absent).
    c. Count number of weevils seen in the quadrat in one minute (use stopwatch).
4. Estimate Vegetation Cover: Use Chart A.
    a. Estimate total vegetation cover (maximum 100%). Write "0" if no vegetation present.
    b. Estimate total cover of rosette garlic mustard. Write "0" if no garlic mustard present.
5. Look for evidence of leaf attack.
    a. Estimate percent of garlic mustard leaf area removed by insect feeding, estimated over the entire quadrat (use Chart A).
    b. Indicate type of damage visible and/or insects present in quadrat: check or write "+" for each type of damage or insect seen.
6. Count the number of garlic mustard rosettes present in the quadrat. If too many to count, estimate density using Chart B.
7. Measure litter depth to the nearest 0.5 cm in the center of each half-quadrat.
8. Looking below all vegetation, estimate perecent cover of bare soil, leaf litter, down wood, and rock. Use Chart A or visually estimate so all 4 categories add up to 100%.
    i. Optional Record presence (and estimated percent cover, if desired) of all plant species rooted in the quadrat. Use Chart A or other scale.
9. After completing Form 3 for quadrat 1, proceed to quadrat 2, and repeat the process (steps 3-9). Continue until all quadrats have been located and recorded.

FORM 3:
GARLIC MUSTARD BIOCONTROL MONITORING (Autumn)

SITE ______________________________ STATE__________
DATE______________________________ GPS___________
Investigators____________________________

please send a copy to: Dr. Bernd Blossey, Fernow Hall,
Cornell University, Ithaca, NY 14853
or fax to: 607-255-0349

Chart A: Percent cover & Damage class

A	<1%
B	1-5%
C	6-25%
D	26-50%
E	51-75%
F	76-95%
G	>95%

Chart B: Estimated Density

I	1-10
II	11-25
III	26-100
IV	100-500
V	>500

Notes:

	Q1	Q2	Q3	Q4	Q5	Q6	Q7	Q8	Q9	Q10	Q11	Q12	Q13	Q14	Q15	Q16	Q17	Q18	Q19	Q20
PERCENT COVER (Use Chart A)																				
Garlic Mustard																				
All other vegetation																				
Leaf Attack (%removed (Use Chart A)																				
Check if present: Leaf miner																				
Windowpane																				
Edge feeding																				
Holes																				
Spittle bug																				
Scale																				
Browse																				
Disease																				
Other																				
ROSETTE DENSITY: (use Chart B if too numerous to count)																				
PERCENT COVER OF: (Use Chart A or actual estimates that total 100%)																				
Soil																				
Wood																				
Leaf																				
Rock																				
LITTER DEPTH (cm)																				
SPECIES PRESENCE or % COVER																				